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Analysis of the Influence of Genotype on Cayenne Pepper Fruit-Receptacle Detachment.

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ANALYSIS OF THE INFLUENCE OF GENOTYPE ON CAYENNE PEPPER FRUIT-RECEPTACLE DETACHMENT

A Dissertation

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in

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DEDICATION

This manuscript is dedicated to my mother, Ruby J. Purvis, who has always been there with love and encouragement, and to my husband, Joe Wayne Gersch, Sr. and children, Charla, Joe Jr., and Amanda, for their support, patience and understanding throughout this study.

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ABSTRACT

Two genotypes of cayenne pepper, *Capsicum annuum* L., were identified that differ significantly in ease of fruit detachment. Both greenhouse and field-grown plants of these genotypes, Cajun 1-9027 and Cap-9004, were investigated for differences in cell type and organization at the fruit-receptacle junction. Scanning electron microscopy revealed that mature fruit of Cajun 1-9027, which did not separate, exhibited a distinct region of sclerified cells that extended from the periphery of the fruit into the receptacle for at least 15 cell layers. In contrast, mature fruit of the more readily detachable Cap-9004 had fewer sclerified cells at the region of detachment. Both histochemical and stereological techniques indicated Cajun 1-9027 had a greater volume of sclereids than Cap-9004. Neither genotype exhibits a well-defined abscission zone at maturity in the detachment region. The presence of more sclerified cells and increased lignification in Cajun 1-9027 compared to Cap-9004 probably contributed to the differences in ease of detachment between the two genotypes.

The usefulness of ethephon, an ethylene releasing compound, as a potential tool in increasing ease of fruit detachment between the receptacle and fruit was also investigated. Ethephon treatments to mature fruit resulted in enhanced maturation, as indicated by fruit color changes, in both genotypes. There were no differences however in fruit detachment force between ethephon treated and untreated fruit.

Examination of sclereid development indicated differentiation by two weeks after anthesis in both genotypes. Sclereid development continued as fruits matured with an increasing trend for both genotypes through nine weeks after anthesis. However, genotypic differences were observed after week nine. In Cajun 1-9027 sclereid volume density increased reaching a sclereid volume density higher than 50% at maturity, thirteen weeks after anthesis. Cap-9004, in contrast, did not increase in sclereid volume density beyond nine weeks after anthesis and sclereid volume density remained near 35%. There were significant differences in sclereid volume density between the two genotypes by thirteen weeks after anthesis.

CHAPTER 1

INTRODUCTION

Cayenne pepper (*Capsicum annuum* L.) fruit are characteristically pendant, long, slender (12.5-25 x 1.9-2.5 cm), thin-walled and pungent (Smith et al., 1987). At the mature stage they are typically red, irregularly shaped and wrinkled, although smooth forms exist.

U.S. production of pungent peppers has increased markedly because of increased use of spices (Smith et al.,1987). The retail processing industry for cayenne hot sauce has a value of 60 million dollars and is expanding (Jim Lusk, personal communication). Several major hot sauce processing companies are located in Louisiana but production of raw product is limited, as most product is imported from other states or countries.

An economical harvest problem with most cayenne pepper fruit used for processing is the tight adherence of the fruit to the receptacle (Smith, 1951). If left attached the woody pedicel and green calyx impart off color and decrease sauce quality. Consequently, processors limit the amount of pepper with pedicel attached to 5% due to quality considerations. Currently, all cayenne pepper for the hot sauce industry is hand harvested. Mechanical harvest is not practical because of the difficulty in fruit removal, as extensive damage to both the fruit and the plant may occur. In addition, hand labor would still be required to remove the calyx from fruit harvested mechanically. Because of the problems associated with mechanical and hand fruit removal of cayenne pepper, a better understanding of pepper fruit detachment is warranted.

Pepper fruit detachment force (FDF) is controlled genetically (Smith, 1951; Spasojevec and Webb, 1971). Smith (1951) crossed the deciduous fruited variety, Chili Piquin, to several nondeciduous varieties and the resulting crosses of the first generation were deciduous. Smith (1951) speculated that the deciduous character is controlled by a single dominant gene. In the segregating populations, however, Smith recognized differences in the force needed to remove the fruit from the stem in the deciduous plants. Spasojevic and Webb (1971) also acknowledged that the phenotypic expression of the gene controlling separation of the fruit from the calyx is variable. They concluded that some dominant genes in the genotypes of certain pepper varieties modify the expression of the dominant effect of the major genes, rendering them incompletely dominant. FDF has also been descriptively correlated to other fruit characters. The correlation of fruit length, width, and weight to FDF occurs in a cross of a banana pepper and a cayenne-type pepper (Werner and Honma, 1980). Other correlations of fruit length and diameter of pedicel and fruit to FDF were reported for most segregating generations of crosses between 'Serrano Chili' (low FDF) and three other cultivars (high FDF); 'Anaheim Chili', 'Keystone Resistant Giant' and 'Red Cherry Small' (Setiamihardja and Knavel, 1990). FDF was measured for separation at both the pedicel-stem (Setiamihardja and Knavel, 1990) and the fruit-receptacle junctions (Werner and Honma, 1980). Some cayenne genotypes detach easier in the fruit-receptacle junction than others (Gersch et

al., 1994; Werner and Honma, 1980). Little is known, however, of the anatomy or histochemistry of the *Capsicum annuum* fruit-receptacle detachment area.

Ethephon, an ethylene releasing compound, has been reported to effectively promote fruit ripening in various crops including pepper (Knavel and Kemp,1973; Love et al.,1971). Research on ethylene-induced color enhancement and pepper fruit ripening has also indicated varying degrees of fruit abscission and defoliation (Sims et al.,1974; Knavel and Kemp, 1973; Cantliffe and Goodwin, 1975; Locascio and Smith, 1977; Batal and Granberry, 1982). Batal and Granberry(1982) reported that ethephon hastened and improved ripening of pimento and paprika peppers and induced defoliation and fruit abscission, especially at later stages of development. Regulating defoliation, flower abscission, immature fruit thinning and abscission of mature fruit provides important crop production control and facilitates harvest. Beaudry and Kays (1988) suggested that selective induction of a particular plant organ requires that the sensitivity of the target organ to the ethylene releasing compound be sufficiently higher than that of nontarget organs. Manipulation of exposure time and concentration give a differential response of olive fruit and leaves to ethylene (Lang, 1989).

Ethephon is approved for use on pepper to promote controlled ripening. Cultivars differ in their response to ethephon (Cantliffe and Goodwin, 1975). Treatments as low as 10 ppm applied twice increase the number of red ripe

fruit in some cultivars (Cantliffe and Goodwin, 1975). Lockwood and Vines (1972) treated pimento peppers with ethephon and noticed an increased rate and higher percentage of red-ripe fruit than those treated with ethylene gas. Armitage (1989) raised the pH from 3.3 to 6.3 and increased the ripening response of larger fruit to the chemical. High concentrations of ethephon accelerated fruit and leaf drop in bell pepper (Knavel and Kemp, 1973) and pimento and chili types (Sims et. al., 1974). Combinations of ethephon and calcium were applied to Tabasco pepper (*Capsicum frutescens* L.) (Conrad and Sundstrom, 1987). Leaf retention was directly proportional to increasing calcium concentrations and inversely proportional to increasing ethephon concentrations. Fruit retention was improved with a 0.1 M calcium concentration. Cooksey et al. (1994) tested different ethephon rates with 0.1 M calcium on paprika pepper (*Capsicum annuum* L.). Increasing ethephon rates increased fruit abscission linearly with or without added calcium. Calcium significantly increased the retention of green fruit on the plant. The effect that ethephon may have on the fruit-receptacle detachment area in cayenne pepper has not been evaluated.

The purpose of the research reported in this dissertation was to investigate fruit detachment in cayenne pepper at the fruit-receptacle junction and to determine the usefulness of ethephon application as a potential tool in ease of detachment between the receptacle and fruit. In addition, anatomical and histochemical studies were performed to further elucidate changes in the fruit-

receptacle detachment area in each genotype. The aim of these studies was to develop an understanding of the genotypic differences in the fruit-receptacle detachment area which results in differential fruit detachment force. This knowledge may contribute to the development of easier methods of hand or mechanical harvest in the future.

Chapter 2 is an extensive literature review which focused on cayenne fruit characteristics and fruit abscission in general. Emphasis was placed on the role of hormones in abscission and the use of plant growth regulators for abscission control. Anatomical and histochemical characteristics of abscission zones were explored with major emphasis on fruit.

The objectives of chapter 3 were to quantify the differences in fruit detachment force and any related fruit characters in selected cayenne pepper genotypes and to evaluate genotypic differences in cell type and organization at the fruit-receptacle detachment area.

Treatments of ethephon were made to fruit of two cayenne pepper genotypes, that differ in FDF, to determine the usefulness of ethephon application as a potential tool in increasing the ease of detachment between the receptacle and fruit (Chapter 4). Concurrently, the anatomical structure of the detachment area was characterized.

The final study was designed to further investigate the genotypic differences in cell type, particularly sclereid development, in the fruit-receptacle detachment area.

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CHAPTER 2

LITERATURE REVIEW

The *Solanaceae* is one of the most important plant families to man (D'Arcy, 1986). This family, also known as the nightshade family, contains five genera of major economic importance in North America. The most important species include: the white potato, *Solanum tuberosum* L.; tomato, *Lycopersicon esculentum* L. Karst; pepper, *Capsicum annuum* var annuum L.; eggplant, *Solanum melongena* L. and tobacco, *Nicotiana tobaccum* L.. Although some 3500 species are known to comprise the family, the distribution of many species is still unknown, and the arrangement of the species into genera and other useful groups is still far from agreed upon. A treatment written at the request of the National Pepper Conference attempts to classify the more important forms of *C. annuum* L. of the United States (Smith, et al., 1987). Members of *C. annuum* L. have the following distinguishing characteristics; flowers white (rarely purple), anthers with anthocyanin, calyx with distinct points or "teeth" and longitudinal ridges, one flower per node (occasionally two at the first flowering node), erect in the wild forms and pendant in many cultivars. Seeds are tan in color. The cayenne group has pendant, long, slender (12.5-25 x 1.9-2.5 cm), medium green, thin walled, pungent fruit, characteristically wrinkled and irregular in shape, although smooth forms are known.

Cayenne pepper was introduced into the United States around 1542 and probably originated in the South American country of French Guiana (Nonnecke, 1989). The pepper was named after the Cayenne River which

flows through that land (Andrews, 1984). From that area it spread throughout the hemisphere and to India and the Orient.

Worldwide production of cultivated pepper, *Capsicum* spp., is increasing in economic importance. There are approximately 51,000 hectares of commercially grown pepper in the United States, of which 25% are pungent types (Petoseed, 1993). In Mexico, there are 83,000 hectares in pepper production; of this more than 95% are pungent. There is a trend of increasing consumption of hot and mildly pungent specialty peppers and products. U.S. production of pungent peppers has increased markedly because of increased use of spices (Smith et al.,1987). In the U.S., the retail processing industry for cayenne hot sauce has a value of 60-70 million dollars and is expanding.

Fruit Quality

Color. Cayenne peppers are harvested when fully red. Chlorophyll, located in the chloroplast thylakoids, reaches a peak in concentration at the mature green stage (Buckle and Rahman, 1979). Ripening processes include chloroplasts turning into chromoplasts. The loss of chlorophyll is not uniform, preventing a consistent color change between mature green and red fruits. The "breaker" or brown color is due to the presence of both chlorophyll and the red pigments (Petoseed, 1993). In the green stage the total carotenoids are low, though cayenne has more beta-carotene, violaxanthin and lutein than other *Capsicum* fruit (Rahman and Buckle, 1979). Lutein and neoxanthin, dominant in the green stage, decrease to very low levels at the ripe stage.

The rise in carotenoids occurs rapidly in the ripening stages from 24 mg/100 g of fruit at mature green to 728 mg/100 g at fully colored ripe stage. The major red carotenoid is capsanthin at 228 mg/100 g of fruit. Other carotenoids present at substantial levels at the fully ripe stage include beta-carotene, zeaxanthin, violaxanthin, capsanthin isomer and capsorubin (Govindarajan, 1991).

Taste and flavor. The flavor is a combination of the volatiles detected by the nose and the taste components sensed by the tongue. Alkyl methoxy pyrazines, which are all highly potent aroma compounds, impart the unique character to the sweet *Capsicum* fruits along with a combination of alcohols, aldehydes, ketones and esters (Govindarajan, 1991). In addition to a unique mixture of these compounds, the pungent varieties, including cayenne, have five capsaicinoids (Petoseed, 1993), which are predominantly capsaicin and dihydrocapsaicin (Govindarajan, 1991). Capsaicin, found on the rib inside the pepper pod, contributes 70% of the pungency (Petoseed, 1993). Cayenne pepper is usually processed into sauce which serves to concentrate and enhance the flavor.

Ease of detachment. Most cayenne, like the other large fruited varieties of *C. annuum*, are non-deciduous. The ripe fruit adheres tightly at the fruit-receptacle junction leaving the green calyx and stem attached when picked. Pepper fruit detachment force (FDF) is controlled genetically (Smith, 1951; Spasojevec and Webb, 1971). FDF is also descriptively correlated to other

fruit characters. Fruit length, width, and weight correlated with FDF in the progeny of crosses of a banana pepper and a cayenne-type pepper (Werner and Honma, 1980). Other correlations of pedicel and fruit length and diameter with FDF were reported in most segregating generations of crosses between 'Serrano Chili' (low FDF) and three other cultivars (high FDF); 'Anaheim Chili', Keystone Resistant Giant' and 'Red Cherry Small' (Setiamihardja and Knavel, 1990). Abscission of reproductive structures in pepper is most frequently caused by environmental factors and appears to be mediated by hormones, particularly ethylene and auxin (Wein, et al. 1989). Plant growth regulators used to increase uniformity of ripening promote abscission of pepper plant organs, including fruit (Batal and Granberry, 1982; Beaudry and Kays, 1988). FDF has been measured for separation at both the pedicel-stem (Setiamihardja and Knavel, 1990) and the fruit-receptacle junctions (Werner and Honma, 1980). Little is known, however, of the anatomy or histochemistry of the *Capsicum annuum* fruit-receptacle detachment area.

Fruit Abscission

Anatomy and Histochemistry. Addicott (1982) defined abscission as "the separation of cells, tissues, or organs from the remainder of the plant body". He explained that the more conspicuous examples are brought about by physiological changes that weaken cell walls and mechanical factors. Esau (1977) pointed out that abscission serves to remove senescent leaves, ripe

fruits, and flowers that did not set, as well as providing a means of self pruning excess shoots (or fruits).

The widespread occurrence of abscission by plants indicates its probable survival advantage and therefore a benefit to the species. Addicott (1982) listed the benefits of abscission as it relates to flower, fruit, and seed: 1) removal of excess flowers, 2) removal of flower parts after function, 3) removal of aborted, diseased or excess fruit, 4) separation of mature fruit and 5) Dispersal of seeds.

Abscission consists of two primary components; separation of the organ from the plant and protection of the remaining tissue. Webster (1973) referred to the work of von Mohl in 1860 as one of the first to note these two sets of phenomena. Discussion of abscission anatomy necessarily requires examination of these processes together and as separate entities. But first, we must define and locate the abscission zone.

Abscission zone (AZ). Bornman et al.(1968) defined the AZ as the "zone in which morphological and physiological changes associated with the abscission of a part or organ are centered". Baird and Webster (1979) reviewed the anatomy and histochemistry of fruit abscission and noted the paucity of relevant literature. Prior to their review, fruit abscission was thought to resemble leaf abscission but, along with the similarities, a number of interesting differences were pointed out. When possible this literature review will refer to fruit abscission at the mature fruit/pedicel junction, but because

the literature is still limited some reference may be made to pedicels, flowers, and immature fruit.

Fruit abscission may be accomplished in different ways. In the same plant, abscission of flowers and immature fruits may differ completely from abscission of mature fruits as McCown (1943) reported for *Malus communis*. Apple flowers and immature fruit abscise following cell division and differentiation of an abscission layer in the basal portion of the pedicel. Mature fruit abscission is initiated independently in the pith and cortex and is not preceded by cell division. *Prunus* species differ in the number and location of AZs. Abscission of sour cherry (*Prunus cerasus* L.) occurs between the fruit and the pedicel (Stosser et al., 1969). The stage of fruit development also affects the abscission of maturing sweet cherry (*Prunus avium* L.). Immature fruit abscise at the upper zone between the pedicel and peduncle and mature fruit abscise at the lower zone between the fruit and receptacle (Wittenbach and Bukovac, 1972). In some *Prunus* species, in which the fruit occurs in clusters, a third AZ may form at the base of the spur (Roth, 1977). One prominent difference between most cultivated and wild *C. annuum* cultivars is the site of the mature fruit AZ. Abscission occurs at the pedicel-stem AZ in the large fruited domesticated *C. annuum* and at the fruit-receptacle AZ in small fruited wild cultivars. Cochran (1936) induced abscission in *C. annuum* (World Beater variety) at the pedicel-stem AZ by applying the following treatments: (1) injecting eosin into the blossom pedicel,

(2) coating young fruits with petroleum jelly, and (3) removing the fruits and leaving varying lengths of pedicel attached to the plant.

The AZ may be easily recognized by external factors; a constriction zone in apple (McCown, 1943) and the fruit-receptacle indentation in the lower zone of maturing sweet cherry (Wittenbach and Bukovac, 1972). In *Cucumis melo* L., both cantaloupe and honeydew differentiate an AZ, which appears as a narrow dark green area, about one week after anthesis at the junction of the pedicel and the fruit (Webster, 1975). In the *C. annuum* pedicel-stem AZ, the first external indication is a slight fading of the green color to a light yellow at the base of the pedicel followed by development of a faint line (Cochran, 1936).

Internal factors also help to identify the AZ. The AZ is structurally similar in cantaloupe and honeydew. The region is composed of 15 - 20 tiers of densely protoplasmic, thin walled, isodiametric parenchyma cells which are distinguishable from adjacent fruit and pedicel cells by their smaller size and by the minimal affinity of their walls for stains (Webster, 1975). The lower AZ in sweet cherry is characterized by progressively smaller cells from the receptacle and fruit sides toward the juncture of the two tissues (Wittenbach and Bukovac, 1971). The juncture is further delineated by the presence of smaller isodiametric cells in the proximal receptacle and angular, expanded cells in the more distal fruit (Wittenbach and Bukovac, 1972). The pedicel-stem AZ in *C. annuum* consists of a band of parenchyma cells, a few cells in

width and distinct from cells proximal or distal to it (Cochran, 1936). The cells in this band have divided at least once, and are smaller and have denser cytoplasm than the surrounding cells.

Chloroplasts frequently decline in number in cortical cells of the AZ of *Phaseolus vulgaris* (Brown and Addicott, 1950) and *Hibiscus rosa-sinensis* (Gilliland et al., 1976). Gilliland et al.(1976) noted that mucilage canals present in the cortex and pith in *Hibiscus* do not extend across the AZ and that the vascular tissues of the AZ are less developed than in adjacent regions. This is not always the case since mucilage ducts cross the AZ in Montbretia (*Crocosmia x crocosmiiflora*)(McKenzie and Lovell, 1992. Webster (1975) observed, in *Cucumis melo* L., the vascular bundles diverge and flare into the fruit at the base of the AZ with no modification of size or shape in the abscission region. In *Phaseolus vulgaris* the amphicribral vascular bundle of the pulvinus divides into the rings of bundles found in the petiole (Brown and Addicott, 1950). Wittenbach and Bukovac (1972) noted the vascular cylinder supplying the fruit branches in the receptacle near the abscission layer and enters the pericarp as 10 - 12 bundles. The only evident weakening of the vascular bundles that they noted is the absence of sclerenchyma associated with the vascular cylinder in the receptacle.

There are widespread reports in the literature that AZs are points of inherent weakness (Sexton and Roberts, 1982). This idea of weakness may have arisen because authors describe a drastic reduction in structural elements

such as lignified fibers, sclerenchyma and stone cells in the AZ compared with adjacent tissues. The zone may not be weak, however, because the structural role of these lignified tissues may be compensated for by an increase in development of collenchyma. Collenchyma has relatively soft, pliable, nonlignified primary walls, whereas sclerenchyma has hard, more or less rigid, secondary walls, which are commonly lignified (Esau, 1977).

Based on a positive reaction to the phloroglucinol-hydrochloric acid stain, Wilson and Hendershott (1968) concluded that lignin is present in cortical and pith cells of *Citrus* fruit AZs on the distal (fruit) side of the separation layer. The pith cells , proximal to the separation layer, are highly lignified and appear to be brachysclereids. Baird and Webster (1979) stated lignin is characteristically absent, or present in only very small amounts, in AZ cells and it is unlikely that parenchyma cells of the cortex of the AZ would undergo lignification. McCown (1943) noted that after abscission of the petals the cells in apple pedicel exhibited wall thickening and evidence of lignification. In spur/pedicel abscission of plum (*Prunus domestica* L.) there is a lack of lignification on the proximal side with cell wall thickening and lignification apparent on the distal side (Simons and Chu, 1975).

Separation layer. Bornman et al., (1967) named the region in the central part of the AZ of cotton, *Gossypium hirsutum,* the separation layer. The separation layer forms by anticlinal cell divisions and consists of a layer of three to six extremely thin-walled cells. Three processes responsible for

separation were observed : (1) cytolysis, (2) dissolution of the middle lamella and (3) dissolution of primary walls (Bornman et al., 1967). Weinheimer and Woodbury (1967) recorded no visible differentiation of the separation layer in potato flower pedicels. Stages of development just prior to abscission show the separation layer to be bounded proximally by the protective layer and on the periphery by the epidermis. Webster (1975) could not predict eventual areas of cell separation in the AZ in cantaloupe. Swelling occurs so randomly it can not be associated with cell separation with certainty. In relationship to the dissolution of cells or cell walls in the separation layer, many researchers, observing various organs and species, noted the vessels and other dead cells rupture mechanically (Gawadi and Avery, 1950; Oberholster et al., 1991; Weinheimer and Woodbury, 1967; McCown, 1943; Wittenbach and Bukovac, 1972).

Abscission does not always follow the same specific pattern of separation but there tends to be a single initiation point. External evidence in cantaloupe is a crack between the pedicel and fruit (Webster, 1975). Internally, preceding separation, disjunct parenchyma cells collapse leaving small cavities within the AZ. Between the cavities are intact parenchyma and vascular tissue which give way to more small cavities. The small cavities eventually coalesce to a single extensive separation cavity. Sexton (1979) related that in *Impatiens sultani,* abscission is induced in a small group of cells found just below the concave groove on the adaxial side of the petiole. As the AZ

weakens, the area of cells showing wall breakdown expand through the parenchyma to the lower side of the stele. The walls of the collenchyma and epidermis along the sides and base of the petiole, and the central vascular tissues, are the last to break down. By examining the AZ in sections, Sexton (1979) reported cell to cell contact is not required for cell wall breakdown to occur at the same time. Apple varieties differ in which tissue abscission is first initiated, with some showing initiation in the pith and others in the cortex (McCown, 1943). Weinheimer and Woodbury (1967) also observed whole regions of cells disintegrate and collapse to form oblong, ragged areas in potato flower pedicels. The first detected cell collapse is located between the vascular system and the epidermis. Pedicel-stem abscission in *C. annuum* starts in the cortex and continues inward (Cochran, 1936). In pedicel abscission of *Hibiscus,* the separation follows yet another pattern with the epidermis showing the first signs of separation and subsequent development proceeding toward the pith (Gilliland et al., 1976). The abscission layer for sweet cherry in the upper zone is well-defined anatomically and histochemically, and indexed by fruit removal force (Wittenbach and Bukovac, 1972). However, abscission occurs at this site only during the fall of immature fruit or after damage or detachment of the fruit or removal of the fleshy pericarp during development. As the fruit approaches maturity there is an apparent strengthening of tissue, resulting in a rise in fruit removal force in the upper zone while the lower zone has a reduction in fruit removal force

leading to abscission. A similar occurrence is noted with orange fruit (Biggs, 1971). The pedicel of *Lycopersicon esculentum* has separation tissue midway between the stem and the flower that serves only for shedding of flowers. If the flower does not separate then the fruits abscise at the pedicel-stem junction (Roth, 1977). The AZ of honeydew is not as well defined as cantaloupe and changes are limited to certain parenchyma cells, which prevent abscission of the fruit (Webster, 1975). In *Nicotiana,* the discolored leaves remain on the plant indefinitely and cell division only occurs to form protective tissue (Gawardi and Avery, 1950).

Protection layer. The protective layer, also called the cicatrice, is formed by deposits of protective substances such as suberin and wound gum (Esau, 1977). Changes take place in several layers of cells on the remaining plant organ to produce resistant protective tissue, prior to or after abscission. After separation of an organ, the cells on the scar surface modify to an extent and become the primary protective layer.

Development of the primary protective layer may involve cell divisions either before or after separation. Addicott (1945) described this as an 'abscission layer' at the leaf base made of dividing cortical parenchyma, resin canal plugs, and cells adjacent to and in the leaf trace. He described suberized cells in this area with an appearance similar to phellem but differentiating from existing parenchyma. In *Lycopersicon esculentum,* if the fruit stalk bends in the flower separation zone and produces a crack in the

separation tissue, a secondary meristem originates from the pith which transforms later into thick-walled lignified cells thought to be a protective layer (Roth, 1977). A concave depression is formed in *C. annuum* pedicel-stem AZ and the surface is protected by the formation of a periderm layer of suberized cells (Cochran, 1936). Weinheimer and Woodbury (1967), in their study of potato, described a crescent shaped protective layer on the remaining pedicel with a thin layer of gums and waxes or a layer of desiccated cells. The degree of development of protective tissue varies in different plants with many developing a secondary protective layer.

The secondary protective layer which occurs in or beneath the primary protective layer usually develops much as periderm and becomes continuous with the adjacent periderm of the stem. McCown (1943) observed in apple that following the abscission of the pedicel or of the peduncle, cork is formed by a cambium which is initiated a few cells below the surface of the scar. Gawadi and Avery (1950) observed, in Poinsettia leaf abscission, that after abscission, the periderm develops in the stem below the scar resulting in a cork layer five to six cells thick. In woody plants the protective layer may develop even more. Rinne et al. (1992) noted that before separation some of the cells on the proximal side of the separation layer become meristematic, repeatedly divide anticlinally, and later die and resemble phellum cells.

Cell Inclusions. Formation of cell inclusions is observed associated with abscission although the relation to abscission is based mostly on speculation.

Brown and Addicott (1950) noticed tyloses develop in the xylem in *Phaseolus vulgaris* . In various treatments on cotton plants, tyloses form in tracheary elements near cut surfaces of the hypocotyl, petiole, and stem and also on either side of the separation layer (Bornman et al.,1967). Oberholster et al. (1991) suggested that restriction of water flow to the soybean pedicel, because of tylose formation, could in part be responsible for abscission. Weinheimer and Woodbury (1967) did not observe tyloses in the xylem elements of potato before or following abscission.

In *Citrus* fruit, starch is present in both cortical and pith cells giving a positive reaction to iodine-potassium iodide (Wilson and Hendershott, 1968). A few starch grains are present in the AZ of *Cucumis* fruit(Webster, 1973). Starch is deposited in the chloroplast of cells proximal to the separation layer in *Hibiscus* (Gilliland et al.,1976). Oberholster et al. (1991) observed large amyloplasts in the bundle sheath cells of the pedicel, with small amyloplasts in the AZ. As abscission continues, the number of large, starch filled amyloplasts in the AZ increases; simultaneously starch is lost in the amyloplasts of the bundle sheath cells of the pedicel. Starch accumulates in the stalk and the AZ of *Phaseolus* explants, treated distally with sucrose, apparently undergoing basipetal carbohydrate movement and sugar to starch conversion (Brown and Addicott, 1950). In the AZ of *Citrus* leaves both calcium oxalate and starch are retained and are present at the time of separation (Scott et al. 1948). Druses of calcium oxalate are numerous in the

parenchyma of the pedicel and the AZ of *Hibiscus* and Gilliland et al. (1976) speculated this is due to calcium release by the hydrolysis of cell wall pectins.

Webster (1975) made the following anatomical observation of cantaloupe which suggests that chemical changes in the walls of AZ cells may accompany structural changes. Staining with ruthenium red to observe pectins in the middle lamella and cell walls indicates: bright red (positive) stain in lamella and walls of parenchyma cells prior to visible structural changes; blue-red stain in swollen wall areas; pink stain in primary walls of isolated parenchyma cells; and no stain in collapsed cell walls.

Physiology. Hormones. Ethylene appears to be the primary regulator of the abscission process, with auxin acting as a suppressor of the ethylene effect (Taiz and Zieger, 1991). Lipe and Morgan (1972), working with three different species (*Gossypium hirsutum* L., *Carya illinoensis* K. Koch, and *Hibiscus esculentus* L.), proposed that ethylene plays a role in regulating natural fruit dehiscence and abscission of young fruit.

Ethylene synthesis begins with methionine which requires ATP combining with sulfate to produce Adenosine 5' phosphosulfate (APS) + PP_i (Taiz and Zeiger, 1991). APS then combines with ATP to produce 3'-Phosphoadenosine 5'-phosphosulfate (PAPS) + ADP. The APS is reduced to sulfide and incorporated into methionine by transsulfuration(primary pathway for higher plants) beginning with serine or homoserine. Sulfate must be reduced prior to assimilation into carbon compounds. The enzymes responsible for this

assimilatory reduction are located in chloroplasts of leaf cells and proplastids of root cortical cells.

Ethylene is formed from methionine via S-adenosyl-L-methionine(AdoMet) and the cyclic, nonprotein amino acid 1-aminocyclopropane-1-carboxylic acid (ACC) (Kende,1993). The enzymes catalyzing the individual steps of this pathway are AdoMet synthetase, ACC synthase and ACC oxidase (EFE). ACC synthase produces, besides ACC, 5'-methylthioadenosine, which is used in the synthesis of new methionine via a modified methionine cycle . This salvage pathway uses one molecule of ATP to form ACC and the methylthio group is preserved through each revolution. High rates of ethylene biosynthesis can be maintained even when the free methionine pool is small. Evidence indicates that ACC synthase is a cytoplasmic enzyme but the cellular location of ACC oxidase is less clear. Many independent experimental approaches indicate compartmentation and membrane association of this enzyme.

Normally ethylene production from plant tissue is low. In some tissues, a large amount of ethylene is produced following trauma caused by chemicals, temperature extremes, waterlogging, drought, radiation, insect damage, disease or mechanical wounding (Yu and Yang, 1980). Ethylene produced by plants under such conditions is referred to as "wound ethylene" or "stress ethylene." Some physiological consequences of stress ethylene are noted. For example, ethylene produced by leaves and fruits, because of drought,

promotes abscission and thereby reduces water loss (McMichael et al., 1972). Mechanical stimulation of ethylene production may include excision, incision, bruising or pressure. Although the biochemistry behind the transduction of mechanical stress into physiological responses is unknown, people have been taking advantage of these phenomena for thousands of years. According to Galil (1968), ripening of the sycomore fig (*Ficus Sycomorus*) of the Middle East can be induced by gashing or piercing immature fruit (about 16 days old) or by treating them with ethylene. The practice of gashing figs is ancient and has been dated back to early Egyptian civilizations. Stress ethylene is produced in Valencia orange (*Citrus sinensis* L.) albedo (Yu and Yang, 1980) and *Citrus unshiu* peel (Shimokawa, 1983) in response to excision. The ethylene production coincides with an increase in ACC content and EFE activity. A similar pattern of events occurred in green tomato (*Lycopersicon esculentum* Mill.) (Yu and Yang, 1980), different ripening stages of tomato (Kende and Boller, 1981) and preclimacteric cantaloupe (*Cucumis melo* L.) (Hoffman and Yang, 1982). Ethylene, ACC, and ACC synthase activity all increased manyfold a few hours after wounding and even after cessation of ethylene production, ACC and ACC synthase levels remain high. Mature green bell pepper fruit (*C. annuum* L. cv. Yolo Wonder) exhibits a non-climacteric pattern of ethylene and carbon dioxide production during normal ripening or exposure to propylene (Saltveit, 1977). However, wounding

excised plugs of ovary wall tissue causes an increase in carbon dioxide production in one day and an increase in ethylene production by day two.

Reid (1985) summarized experimental evidence to support the role of ethylene in natural abscission. His synopsis includes: 1) Ethylene production increases prior to abscission in many abscising organs. 2) Treatment of a wide range of plant species with ethylene or with ethylene-releasing compounds stimulates abscission. 3) Silver ion, a potent and apparently specific inhibitor of the action of ethylene, inhibits not only ethylene stimulated abscission but also abscission caused by other stresses. Reid (1985) also recapitulated increasing support for the view that changing sensitivity to plant hormones is at least as important as changes in their endogenous concentrations as a control mechanism.

In studies of environmental conditions and applied plant hormones (plant growth regulators), Reid (1985) recounted the following consistencies: 1) A gradient of auxin from the subtended organ to the plant axis maintains the AZ in a nonsensitive state. This gradient is maintained by factors such as auxin, cytokinins, light, and good nutrition which inhibit senescence of the organ. 2)Reduction or reversal of the auxin gradient causes the AZ to become sensitive to ethylene. 3) Once sensitized, the cells of the AZ respond to low concentrations of ethylene, whether exogenous or endogenous, by the rapid production and secretion of hydrolytic enzymes and subsequent shedding of the subtended organ.

Enzymes. In both leaves and fruit, all levels of dissolution of pectic and other cell wall constituents, based on histochemical studies, are reported, including complete cytolysis (Baird and Webster, 1979). The dissolution of cell wall polysaccharides requires the activity of multiple cell wall hydrolases, each potentially responding to a unique subset of developmental, hormonal, and environmental cues (Lashbrook et al., 1994). The involvement of endo B-1,4 glucanases (EGases; B[1:4] 4-glucan hydrolase; EC 3.2.1.4) is implicated in hemicellulose degradation. Plant EGases lack cellulose binding domains so there is no structural evidence that plant endoglucanases are capable of degradation of crystalline cellulose. The term "cellulase" has been widely used to describe these endoglucanases, however, the term is misleading in view of the current lack of evidence for EGase-catalyzed cellulose degradation.

Polygalacturonase and cellulase activity are reported in AZs of many fruits which suggests that the breakdown of cell wall constituents is caused by these enzymes (Huberman and Goren, 1979; Greenberg, et al., 1975; Bonghi et al., 1993). The establishment of EGase activity in abscising tomato flowers (Roberts et al., 1984) and studies of EGase gene expression in bean abscission (Tucker et al., 1988) suggests a role for EGases in cell wall dissolution processes (Lashbrook et al., 1994). Cel1, a member of a multigene EGase family in tomato, accumulates in flower AZs. This accumulation is consistent with the observation of significant amino acid sequence identity (68%) between tomato Cel1 and bean AZ EGase. Research

on cellulase in the bean plant, *Phaseolus vulgaris* L. led to the discovery of 9.5 cellulase (Durbin and Lewis, 1988). This basic cellulase is synthesized *de novo* in response to ethylene and is inhibited by auxin. Synthesis of this form of cellulase is limited to a narrow band of cells in the AZ. In bean, it appears to function in shedding of various organs such as fruits, flowers and leaves. Cellulase is also localized in the cell separation layer of *Gossypium hirsutum* L. and *Coleus blumei* explants AZ (Abeles, 1969). Cellulase activity is markedly increased before abscission and for a period after excision in citrus leaf explants (Ratner et al., 1969). Riov (1974), in a study with citrus leaf explants, concluded that polygalacturonase, in addition to cellulase, plays a role in abscission. The polygalacturonase (PG; poly-1,4-D-galacturonide glycanhydrolase, EC 3.2.1.15) from citrus leaf explants is an exopolygalacturonase and appears to be a soluble enzyme. Berger and Reid (1979) examined the role of PG in the AZ of leaves of *Phaseolus*. The results show that PG is localized in the AZ, although changes in enzyme activity are not found to accompany abscission.

Greenberg et al. (1975) suggested the dissolution of cell wall components and middle lamella occurs at about the same time in *Citrus* fruit due to the similar behavior of cellulase and PG and parallel patterns of the rise in their activity. The activity of the enzymes is also suppressed by auxin and increased by ethylene.

Ethylene induced abscission in leaf and fruit explants of peach involves different enzymes (Bonghi et al., 1992). The level of cellulase activity is higher in leaf abscission than in abscising fruit explants. PG activity is high in ethylene induced fruit explant abscission but not detectable in abscising leaves. These differences in enzyme pattern and induction suggest the feasibility to regulate peach fruit abscission with the aid of antisense RNA genes.

Other enzymes with localized activity in AZ include dehydrogenase, acid phosphatase and peroxidase in both sweet and sour cherry (Pooviah et al., 1973). Peroxidase activity increases in ethylene treated tobacco AZs and correlates to a decline in break strength (Henry et al., 1974). The increased localized activity of these additional enzymes is indicative of an association with abscission though the causal role is not clear. Pooviah et al.(1993) suggest that peroxidase may regulate endogenous levels of indoleacetic acid in fruit abscission.

Temperature response. The temperature response for pepper plants (*Capsicum annuum* L.) undergoing heat induced flower abscission has been studied for two cultivars (Aloni et al. 1994). The curve shows maxima near 48°C for the bell pepper cultivar (Maor) and maxima near 30°C for the paprika cultivar (Lehava). This temperature data indicates that the process of abscission has cultivar dependent chemical reactions that are an important and limiting step.

Oxygen response. Oxygen relations were investigated with cotton explants (Marynick and Addicott, 1976). The data of the abscission response to O_2 takes the form of a double sigmoid curve. The rate of abscission rises sharply as O_2 increases to 10 %. There is a plateau between 10 and 20%, followed by a sharp rise at about 25% O_2 to the maximum rate at about 30%. The rapid rise in rate at low levels of O_2 agrees with other physiological processes that are respiration limited. The acceleration of abscission by O_2 levels above 20% has not been examined. A possibility exists that high O_2 may stimulate ethylene production, as one of the final steps in ethylene synthesis is O_2 dependent (Liberman, 1979). Because of this oxygen requirement and the inhibition of abscission by most respiratory inhibitors, respiration is thought to be involved in abscission.

Nutritional factors. High carbohydrates in a plant will contribute to the vigor of fruits and leaves, and in general will enable such organs to readily synthesize the hormones required for growth, development, and inhibition of abscission. Plant tissues that are high in nitrogen are well supplied with amino acids and other nitrogenous compounds essential for active metabolism. Vigorous organs delay their own abscission by exporting to their AZ greater amounts of auxin (Avery et al., 1937; Avery and Pottorf, 1945).

Abscission of leaves and sometimes fruits is one of the recognized symptoms of deficiencies of the mineral elements. Calcium, a major constituent of the cell wall, when deficient can promote abscission and when

applied can retard abscission (Pooviah and Leopold, 1973; Conrad and Sundstrom, 1987).

Stress factors. Stresses that prevent vigorous healthy growth also seem to promote abscission. Wien et al. (1989) stated that the most frequent causes of abscission of pepper reproductive structures are environmental factors such as heat, drought or low light conditions, diseases or insect pests. When at least some of these stresses are present, abscission appears to be mediated by ethylene generated within the reproductive tissue.

Genetics. Evidence of genetic control of abscission is widespread, although it is not well documented. McCown (1943) reported varietal differences in apple in which tissue abscission was first initiated and whether the process was limited to the constriction zone. The potato variety Russet Burbank abscises many more of its flowers and young fruit than does the variety Menominee (Weinheimer and Woodbury, 1967). Wide pod detachment force exists between 16 southern pea cultivars (Buckley and Pee, 1994). Smith (1951) crossed the deciduous fruited variety, Chili Piquin, to several nondeciduous varieties and recorded the resulting crosses of the first generation were deciduous. He speculated that the deciduous character is controlled by a single dominant gene although in the segregating populations he recognized differences in the deciduous plants in the force needed to remove the fruit from the stem. Spasojevic and Webb (1971) also acknowledged the phenotypic expression of the gene controlling separation

of the fruit from the calyx is variable. They assumed that certain dominant genes in the genotypes of certain pepper varieties modify the expression of the dominant effect of the major genes, rendering them incompletely dominant.

Summary of abscission. At the junction of most discrete plant organs with the remainder of the plant is an AZ. The external and internal appearance of this zone is usually readily distinguished from surrounding tissue. The development of an AZ varies from early ontogeny in some species to just before abscission in others(Webster, 1975; Brown and Addicott,1950). The presence of an abscission region does not always indicate separation will occur (Webster, 1975; Rinne et al.,1992) nor does the absence indicate it will not (Webster, 1973). The amount of development of the AZ varies from species to species and depends on the conditions under which it occurs (Gawadi and Avery,1950; Bornman et al., 1967). The degree of differentiation is particularly interesting because it bears on the relative ease with which fruits separate naturally or are separated mechanically and apparently has no significance in leaf separation(Baird and Webster, 1979). Many of the later studies are based on classical work done in the late 1800's and early 1900's in which descriptions were important to developing systems of classification (Webster, 1973). Recent papers are more concerned with the manner in which separation takes place and the changes that take place on a cellular level. Recent work combines anatomical and histochemical studies

with as many questions left open as are answered. A general consensus appears to exist, in more defined AZs, for the division into a separation layer and a protective layer. The separation layer is involved in actual separation of the organ from the plant. The protective layer that develops, before or after separation, prevents desiccation or entry of foreign organisms to the remaining plant. The use of growth regulators to retard and stimulate abscission is another active area of study and will be the focus of one area of this research.

Plant growth regulators

The knowledge of action and lack of control of the major endogenous plant growth regulators (PGRs) has led to extensive study of exogenously applied chemical PGRs. Most of the major plant hormones have chemical analogs. Application of these PGRs may be in the form of a gas (Wien et al., 1989; Lang and Martin, 1989), a spray (Wien and Zhang, 1991; Bukovac et al., 1971), by means of a wick (Wien et al., 1989), as a paste (Singh and Murty, 1983; Wien and Zhang, 1991) or other means that better suit the experimental purpose and the chemicals known action. In this study the major chemical of interest is one known to release ethylene, 2-chloroethylphosphonic acid (ethephon, CEPA, "Amchem 66-32", "Ethrel"). Ethephon degrades to yield ethylene in an alkaline solution and has been very effective in causing responses characteristic of ethylene treatment (Yang, 1969). The most probable course of action involves a water molecule,

probably the hydroxide ion, reacting with ethephon to produce ethylene, chloride, and phosphate.

The practical uses of ethephon related to flowering, vegetative growth and dormancy, abscission, ripening and maturity, disease and freeze resistance, and latex flow are well known and have been commercially important for over 25 years (de Wilde, 1971). Regulating defoliation, flower abscission, immature fruit thinning and abscission of mature fruit provides important crop production control and facilitates harvest. The removal of leaves in some crops, such as cotton and snap beans, facilitate harvest and is desirable but in crops not suitable for once over harvest, or fruit crops grown on trees, leaf removal may be detrimental to the plant or future harvests (Beaudry and Kays, 1988). Beaudry and Kays (1988) suggested selective induction of a particular plant organ requires that the sensitivity of the target organ to the ethylene releasing compound be sufficiently higher than that of nontarget organs. Manipulation of exposure time and concentration give a differential response of olive fruit and leaves to ethylene (Lang, 1989). Lowering the pH to 3 decreases leaf drop and does not effect olive fruit removal force (Denny and Martin, 1994).

Ethephon is approved for use on pepper to promote controlled ripening and studies date back to the early 1970's (Love et al., 1971; Sims et al., 1974). Cultivars differ in their response to ethephon (Cantliffe and Goodwin, 1975). Treatments as low as 10 ppm applied twice increase the number of red ripe

fruit in some cultivars. Lockwood and Vines (1972) treated pimento peppers with ethephon and noticed an increased rate and higher percentage of red-ripe fruit than those treated with ethylene gas. Armitage (1989) raised the pH from 3.3 to 6.3 and increased the ripening response of larger fruit to the chemical. High concentrations of ethephon accelerate fruit and leaf drop in bell pepper (Knavel and Kemp, 1973; Locascio and Smith, 1977) and pimento and chili types (Sims et. al., 1974). Combinations of ethephon and calcium were applied to Tabasco pepper (*Capsicum frutescens* L.) (Conrad and Sundstrom, 1987). Leaf retention was directly proportional to increasing calcium concentrations and indirectly proportional to increasing ethephon concentrations. Fruit retention was improved with a 0.1 M calcium concentration. Cooksey et al. (1994) tested different ethephon rates with 0.1 M calcium on paprika pepper (*Capsicum annuum* L.). Increasing ethephon rates produced a linear increase in fruit abscission with or without added calcium. Calcium significantly increased the retention of green fruit on the plant.

Environmental factors such as temperature, humidity and light intensity significantly alter the release of ethylene from ethylene releasing compounds (Beaudry and Kays, 1987). The effects of these factors cause differential responses for time of application with peppers.

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CHAPTER 3

ANATOMICAL DESCRIPTION OF THE FRUIT-RECEPTACLE DETACHMENT AREA IN CAYENNE PEPPER

Introduction

Worldwide production of cultivated pepper, *Capsicum spp.*, is increasing in economic importance. There are approximately 51,000 hectares of commercially grown pepper in the United States, of which 25% are pungent types (Petoseed, 1993). In Mexico, there are 83,000 hectares in pepper production; of this less than 5% are sweet (primarily for export). There is a trend of increasing consumption of hot and mildly pungent specialty peppers and/or pepper products. U.S. production of pungent peppers has increased markedly because of increased use of spices (Smith et al.,1987). In the U.S., the retail processing industry for cayenne hot sauce is 60-70 million dollars and expanding (Petoseed, 1993).

Pepper harvesting is often difficult and a major expense in production. In most of the cultivated pepper types of *Capsicum annuum* L., including cayenne, the fruit adheres tightly to the receptacle leaving the calyx and pedicel attached when harvested. Tight adherence of the fruit to the receptacle is an important characteristic for hot sauce as the woody pedicel and green calyx impart off-color and decrease sauce quality. Consequently, processors limit the amount of pepper with pedicel attached to 5% due to quality considerations. Currently, all cayenne pepper for the hot sauce industry is hand harvested. Mechanical harvest is not practical because of the difficulty in fruit removal, as extensive damage to both the fruit and the plant may occur. In addition, hand labor would still be required to remove the calyx

from fruit harvested mechanically. Because of the problems associated with mechanical and hand fruit removal of cayenne pepper, a better understanding of pepper fruit detachment is warranted.

Pepper fruit detachment force (FDF) has been reported to be controlled genetically (Smith, 1951; Spasojevec and Webb, 1971). FDF also has been descriptively correlated to other fruit characters. Fruit length, width, and weight correlated with FDF in progeny of crosses of a banana and a cayenne-type pepper (Werner and Honma, 1980). Other correlations of length and diameter of pedicel and fruit to FDF were observed in most segregating generations of crosses between 'Serrano Chili' (low FDF) and three other cultivars; 'Anaheim Chili', Keystone Resistant Giant' and 'Red Cherry Small' (high FDF) (Setiamihardja and Knavel, 1990). Abscission of reproductive structures in pepper is most frequently caused by environmental factors and appears to be mediated by hormones, particularly ethylene and auxin (Wein, et al. 1989). Plant growth regulators used to increase uniformity of ripening have promoted pepper fruit abscission (Batal and Granberry, 1982; Beaudry and Kays, 1988). Pepper FDF has been measured for separation at both the pedicel-stem (Setiamihardja and Knavel, 1990) and the fruit-receptacle junctions (Werner and Honma, 1980). Little is known, however, of the anatomy or histochemistry of the *Capsicum annuum* fruit-receptacle detachment area. The objectives of this study were to quantify the differences in fruit detachment force and any related fruit characters in

selected cayenne pepper genotypes and to evaluate genotypic differences in cell type and organization at the fruit-receptacle detachment area.

Materials and Methods

Screening genotypes for differential FDF. Greenhouse studies. On 18 Dec.,1992, eight cayenne pepper genotypes (C 19-9008, C 19-9012, Cap-9004, C 16-9006, Cap-9016, Durk-9026, Cajun 1-9027, and Cajun 1-9041) were sown in 72 cell trays with a commercial medium (Metro Mix 200; Grace Sierra, Milpitas, Calif.). These genotypes were selected and loosely matched on characteristics of size of calyx, shoulder area and difference in ease of detachment by hand-testing during previous field observations. Seedlings were transplanted on 29 Jan., 1993, into 11.3 liter pots filled with a modified commercial medium (Metro Mix 700; Marysville, Ohio) supplemented with micronutrients (Micromax; Grace Sierra, Milpitas, Calif.) 780 mg/liter, dolomitic limestone (Easy Lime; Sylacauga, Ala.) 6.26 g/liter, and slow release fertilizer (Osmocote 14N-6.2P-11.6K; Scott Sierra, Marysville, Ohio) 4.7 g/liter. The plants were grown under natural daylight with daily irrigation and standard cultural practices for pest control (Boudreaux et al.,1992). The plants were placed on benches 1 m apart in staggered double rows arranged in a randomized complete-block design with five blocks. Two fruit were randomly harvested from each plant weekly for five weeks, beginning 18 May, 1993. Fruit were sampled at the mature red stage with some pericarp wrinkle.

Statistical analyses were performed on the means from each plot for each harvest.

Fruit Detachment Force (FDF) was measured using a force gauge graduated in 25 g increments up to 2.5 kg (Chatillon and Sons, Kew Gardens, N. Y.). The force gauge was modified according to Werner and Honma (1980) and attached to a stand. The pedicel was firmly clamped proximal to the calyx between the steel bar and adjustable bar on the clamp. FDF was determined by slowly pulling the fruit perpendicular to the longitudinal axis of the pedicel-fruit system until the fruit detached from the pedicel. Force was recorded in kilograms and converted to Newtons. Other characters measured included: fruit length and diameter, pedicel length, calyx diameter, length, and scar. Fruit length was measured as the distance from pedicel attachment at the calyx to its apex. Fruit diameter was measured at its maximum width. Pedicel length was measured as the distance between the points of attachment to the stem and to the calyx. Calyx diameter was measured at the distal end of the calyx. Calyx length was measured from the point of attachment to the pedicel to the distal end. The scar was measured on the fruit after the calyx was removed. All measurements were made with vernier calipers except fruit length which was measured with a metric ruler. Correlations were performed for each genotype between these characters and FDF.

Field studies. Based on the results of the preliminary greenhouse study, four varieties were selected for the field study. Two hard detaching (Cajun 1-9027, Cajun 1-9041) and two easy detaching genotypes (Cap-9004, Durk-9026) were seeded on 22 March, 1993 and transplanted on 10 May, 1993 into aluminum painted black plastic mulched plots. Plots consisted of three plants of each genotype arranged in a split-plot design with genotypes as the main plot (in four complete blocks) and four harvest dates as subplot treatments. Standard cultural practices (Boudreaux et al.,1992) were followed. To sequence fruit maturity, all mature red fruits were removed and after three days the remaining fruit that had turned red were tagged. The tagged fruits were harvested four days after tagging. The fruit were carefully removed from the stem leaving the pedicel intact. The first harvest was 10 Aug, 1993. Four of the harvested fruit were randomly selected from each plot for determination of FDF. The means from each plot were used in the statistical analysis.

Anatomical and Histochemical studies. Plant material. Fruits of two cayenne pepper genotypes, Cajun 1-9027 and Cap-9004, were collected from field grown plants in 1993 and greenhouse grown plants in 1994. Cajun 1-9027 fruit do not separate and Cap-9004 separates with applied force at the fruit-receptacle junction. Fruit were tagged and harvested as in the field experiment above. Samples were randomly selected from these harvests for anatomical and histochemical studies. To quantify the location of the

detachment area at the fruit-receptacle junction, 223 peppers of Cap-9004 were hand separated and the area of separation was observed.

Anatomical studies. Longitudinal tissue samples, approximately 0.6 cm x 0.3 cm, were prepared to include the region of future detachment, located at the fruit-receptacle junction. Approximately equal portions of fruit and pedicel were included. Samples were placed immediately into 5:5:90, 37% formaldehyde: glacial acetic: 70% ethanol (FAA), by volume, and subjected to a mild vacuum for 24-48 hours.

Light microscopy (LM). FAA- fixed samples were dehydrated and embedded in paraffin using standard techniques (Jensen, 1962). Sections were cut at 5-10 μm using disposable blades on a rotary microtome. To analyze general cellular structure, sections were stained with 0.5% toluidine blue or 1.0% safranin counterstained with 1.0% alcian blue. For histochemical studies, slides were stained with ruthenium red for pectin localization, periodic acid-Schiff's (PAS) reagent for detection of insoluble polysaccharides, iodine-potassium iodide (IKI) to observe starch distribution, and phloroglucinol and HCl for lignin detection (Jensen, 1962). Polarized light was used in addition to the last two tests (Berlyn and Miksche, 1976).

Scanning electron microscopy (SEM). FAA fixed samples were dehydrated with ethanol. The samples were critical point dried with CO_2, mounted on Al stubs with double sided tape, and sputter coated with 20 nm of gold

palladium. Observations and photographs were made on a Leica Cambridge stereoscan 260 scanning electron microscope operated at 8 kV.

Quantitative analysis. Stereological techniques were applied to quantitate volume densities of the various cell types in the two genotypes (Toth,1982). Four fruits of each genotype were randomly sampled and tissue sections prepared as described in LM. Photomicrographs were made with a final magnification x50. A transparency, marked with boundaries equivalent to 2 mm on either side of the fruit-receptacle indentation distal to the pedicel, was secured to the photomicrograph. A second transparency with a grid was randomly placed on the photomicrographs and point counts recorded. Volume densities of parenchyma, sclerenchyma, vascular tissue, and intercellular spaces were calculated from the point counts. Volume densities of the various cell types in the two genotypes were compared with the z statistic, $P < 0.05$.

Results

Screening genotypes for differential FDF. Greenhouse studies. Fruit detachment force varied considerably between the eight genotypes. The variation in FDF ranged from high (> 24.5 N, fruit would tear before detachment occurred) to low (< 19.6 N) values. Some genotypes had intermediate FDF and other genotypes had extreme variation over the four harvests (data not shown). Differences in rate of maturation reduced the number of red fruit available from the first harvest, therefore only the second

through fifth harvests were included in the analysis. Four of the eight genotypes were selected for further study: two (Cajun 1-9027 and Cajun 1-9041) with high FDF values and two (Cap-9004 and Durk-9026) with low FDF values (Fig.1). Contrasts indicated significant differences between the two genotypes with high FDF when compared with the two with low FDF for all four harvests. Contrasts also indicated the FDF for Cap-9004 was significantly lower than Cajun 1-9027 for all four harvests.

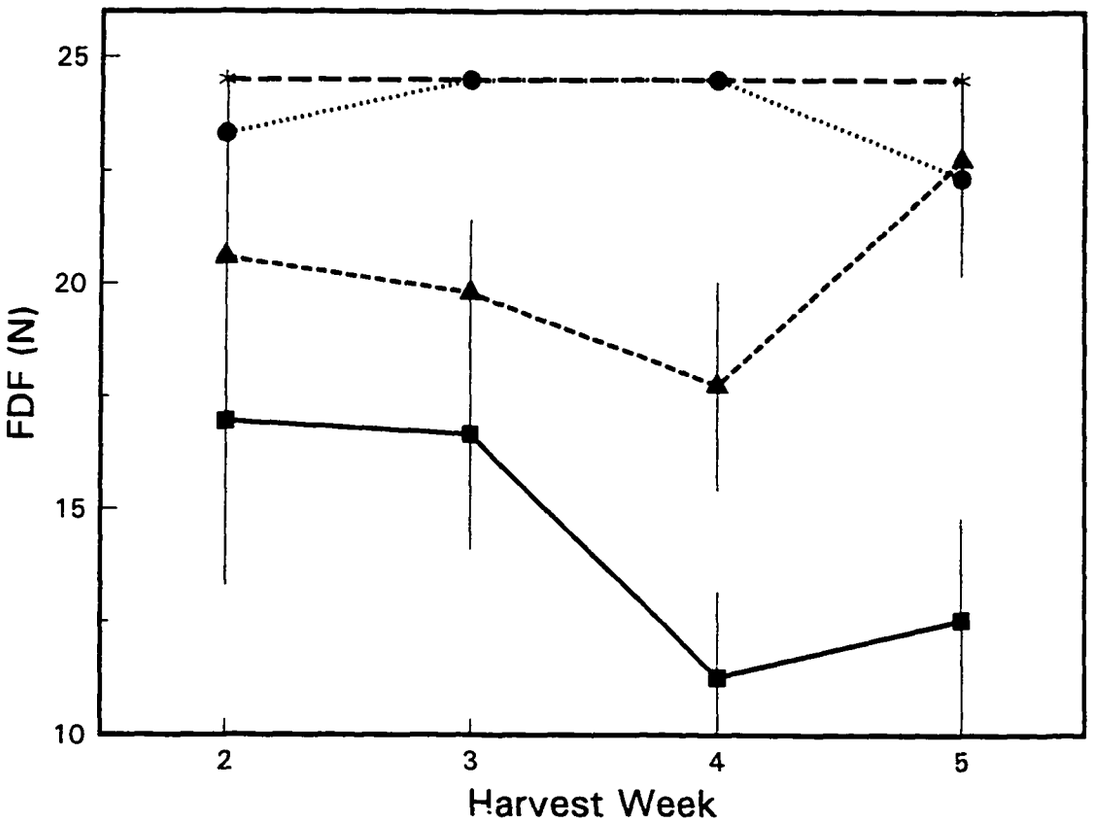

Fig. 1. Fruit Detachment Force (FDF) of four cayenne pepper genotypes for four harvests in the greenhouse. Cap-9004 (■), Durk-9026 (▲), Cajun 1-9027 (●), Cajun 1-9041 (*). Vertical bars represent SE of the means; error bars that do not appear on graphs are smaller than symbols.

Linear correlations of FDF with fruit characters were examined for the five genotypes that varied in FDF (Table 1). No significant correlations between FDF and fruit characters were consistent over the five genotypes examined. There was, however, a significant correlation of FDF to fruit length for Cap-9004. FDF was also significantly correlated to pedicel length and fruit diameter for C 19-9012. No significant correlations were observed between FDF and calyx characters for the five genotypes studied.

Table 1. Correlation coefficients for association of fruit detachment force with fruit length or diameter, calyx length, diameter, or scar, and pedicel length for five genotypes that varied in FDF.

Genotype	Fruit		Calyx			Pedicel length
	Length	Diam	Length	Diam	Scar	
Cap-9004	0.39**	0.09	-0.12	-0.12	-0.09	-0.05
C 16-9006	0.11	-0.28	-0.03	-0.17	0.29	-0.03
C 19-9012	0.27	0.34*	-0.18	0.28	0.02	0.38**
Cap-9016	0.00	0.17	0.07	-0.20	-0.10	0.00
Durk-9026	-0.07	0.05	0.15	-0.19	0.10	-0.06

*,** Significant at P = 0.05 and 0.01 respectively.

Field studies. FDF values varied over the harvest period, with differences apparent for harvests two, three, and four (Fig. 2). Contrasts of the two high FDF genotypes (Cajun 1-9027 and Cajun 1-9041) with the two low FDF (Cap-9004 and Durk-9026) indicated significant differences for all four harvests. In addition, contrasts indicated FDF values for Cap-9004 and Cajun 1-9027,

two genotypes that had been matched on fruit characters, were significantly different for harvests two through four.

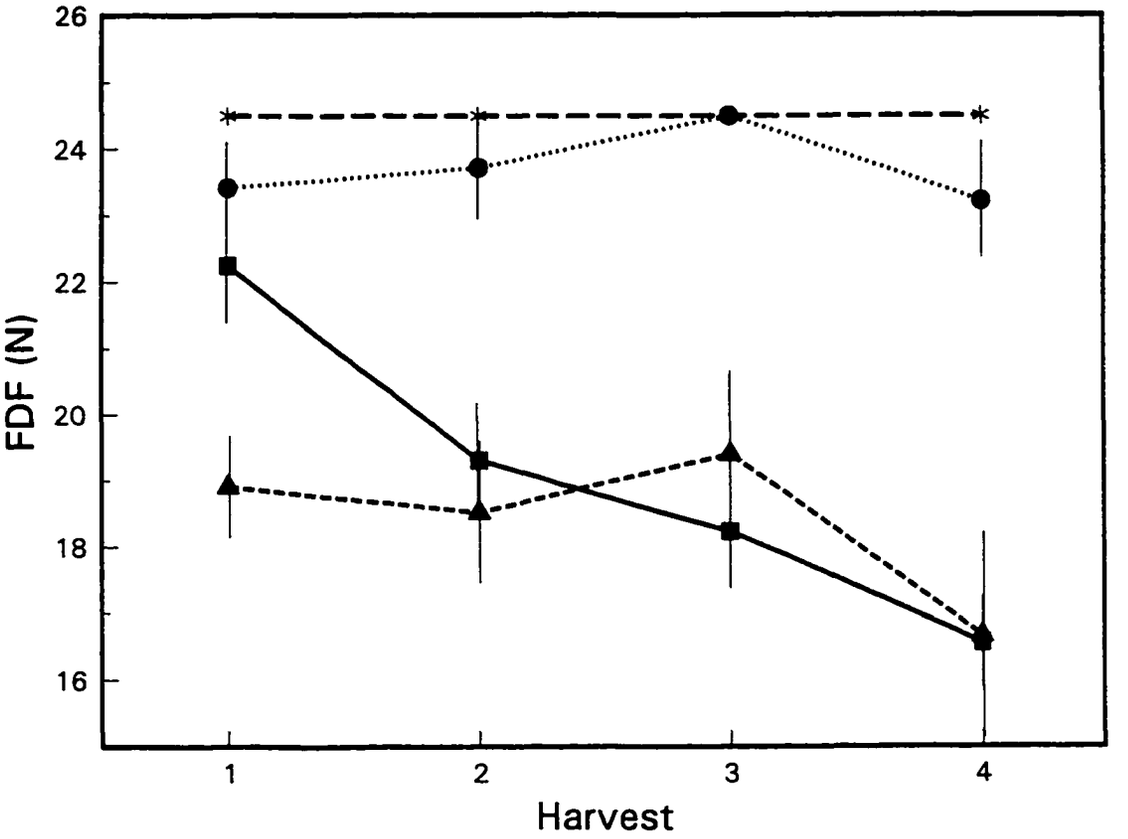

Fig. 2. FDF of four cayenne pepper genotypes for four harvests in the field. Cap-9004 (■), Durk-9026 (▲), Cajun 1-9027 (●), Cajun 1-9041 (*). Vertical bars represent SE of the means; error bars that do not appear on graphs are smaller than symbols.

Anatomical and Histochemical Studies. Anatomy of detachment area. The fruit-receptacle detachment area was delineated externally by the fruit-receptacle indentation (Fig. 3). The area of study included a region 2 mm into both the fruit and receptacle from the indentation. The vascular cylinder supplying the fruit branched in this region and entered the fleshy pericarp.

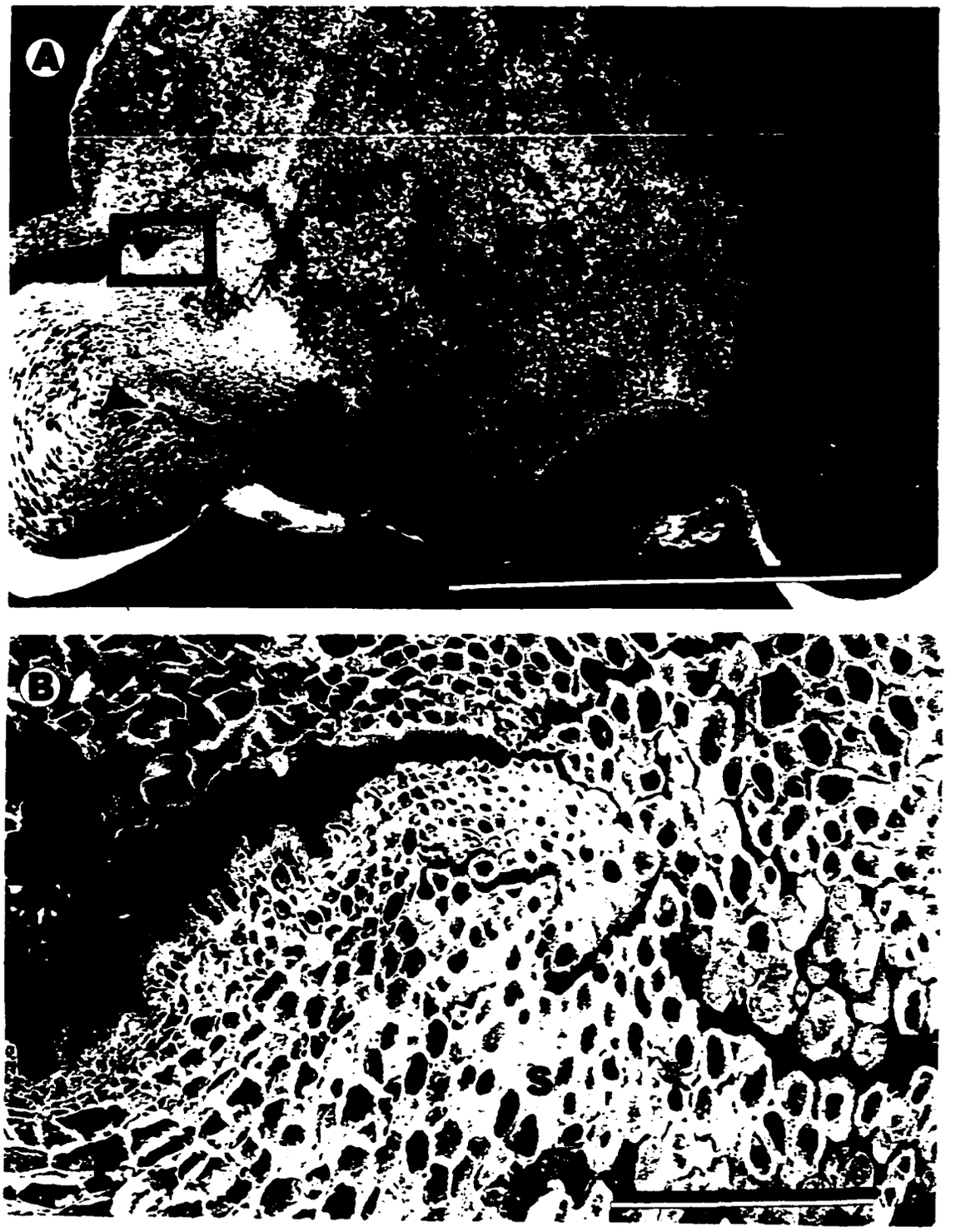

Fig. 3. SEM images illustrating the fruit-receptacle junction in Cajun 1-9027. (A) longitudinal tissue section, line represents 5 mm. (B) enlargement of fruit-receptacle indentation outlined in A. Abbreviations: receptacle (R), fruit (F), vascular tissue (V), indentation (I), sclerenchyma (S), parenchyma (P). Line represents 200 μm.

Of the 223 fruit of Cap-9004 separated, to characterize where detachment takes place, 25% separated clean (little, or no fruit left attached) at the fruit-receptacle junction just distal to the fruit-receptacle indentation (Fig. 4). Over half, 55%, separated proximal (in this case, xylem strands were broken and protruding) to the fruit-receptacle junction in varied positions, from just proximal, to leaving a portion of the pedicel attached (data not shown). The remaining 20% separated either distally, with fruit tissue attached, or fruit detached unevenly. Other than the clean detachment, no other detachment appeared consistent.

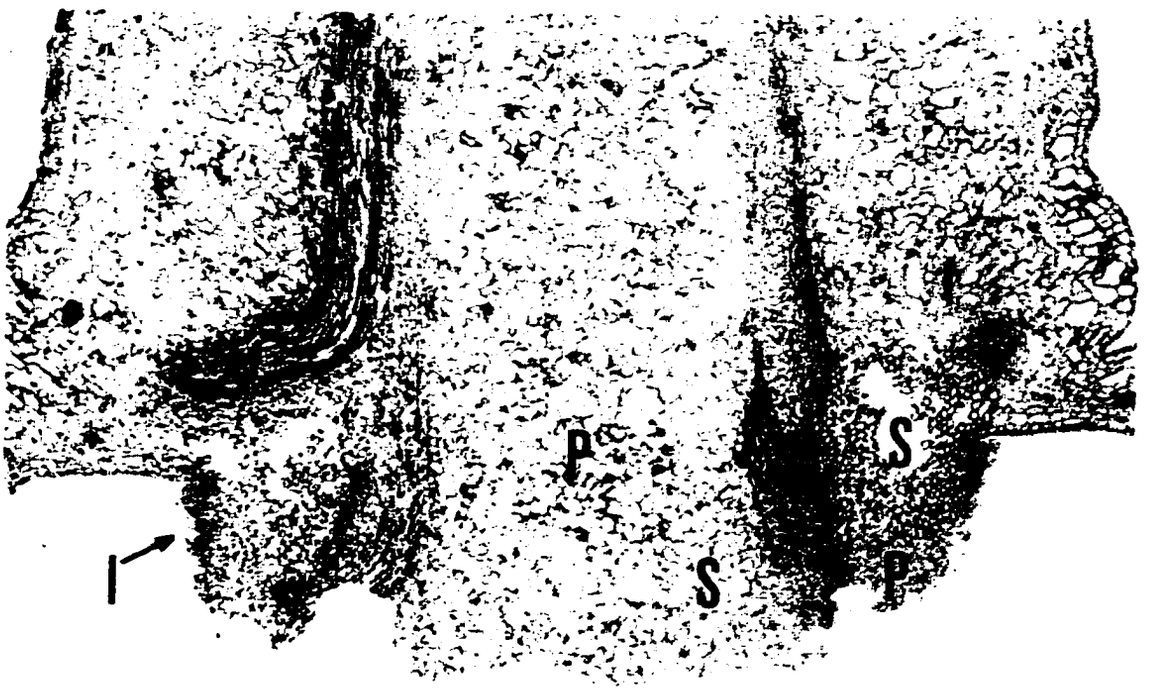

Fig. 4. Photomicrographs of longitudinal sections of separated cayenne pepper fruit stained with 1.0% safranin and counterstained with 1.0% alcian blue. Cap-9004. Abbreviations: indentation (I), parenchyma (P), and sclerenchyma (S). Magnification x15.

Scanning electron microscopy revealed that mature fruit of Cajun 1-9027, which does not separate, exhibits a distinct region of sclerified cells that extend from the periphery of the fruit into the receptacle for at least 15 cell layers (Fig. 3b). In comparison, mature fruit of the more readily detachable genotype, Cap-9004, had fewer sclerified cells at the point of detachment (not shown).

Light microscopy revealed the transition region between the fruit and receptacle outside the vascular cylinder was characterized by progressively smaller cells from the receptacle and fruit toward the juncture of the two tissues in both genotypes. In Cajun 1-9027, the cells in the transition region were sclerified and continued from the cortex (approximate size, 30-38 μm) through the pith (25-49 μm) to form a disk of small sclerified cells that extended from the receptacle into the fruit (Fig. 5a). Cells above the transition region in both the cortex (73-107 μm) and the pith (67-105 μm) were similar in size to cells below this region in the cortex (60-96 μm) and pith (58-98 μm). The cells in these areas were larger than cells in the transition region. Cap-9004 also exhibited small sclerified cells in the transition region that were mainly confined to the cortex (20-32 μm) of the fruit-receptacle area (Fig. 5b). The sclerified cells in the pith of Cap-9004, however, were not continuous with the sclereids in the cortex and the cells in the pith (59-117 μm) were larger than sclereids in the cortex with no apparent reduction in size from adjacent cells in the receptacle (76-122 μm) and fruit (68-116 μm) (Fig. 5b).

Histochemistry of detachment area. Localization data for insoluble polysaccharides and pectins revealed no change through the detachment area of either genotype in comparison to the adjacent fruit and receptacle tissue. Cajun 1-9027 stained intensely in the detachment area and surrounding fruit tissue in both tests indicating a dense cell structure. In comparison, Cap-9004 stained less intensely in this region suggesting a less dense cell structure.

Localization of starch indicated little or no starch in the detachment area. Starch was, however, present in the receptacle and the fruit tissue of both genotypes.

Lignified cells were present throughout the fruit-receptacle area in Cajun 1-9027. There was less lignification in the detachment area in Cap-9004. Both sclereids and xylem cells gave a positive reaction for lignin with phluoroglucinol and HCl and polarized light indicated thickened secondary cell walls (Fig. 6).

Quantitative Composition. The volume density calculated for parenchyma was significantly greater ($P = 0.001$) in Cap-9004 (.581) compared with Cajun 1-9027 (.484) (Table 2). In comparison, the volume density calculated for sclerenchyma was significantly greater ($P = 0.001$) in Cajun 1-9027 (.409) than in Cap-9004 (.294). There was no significant difference in volume density of vascular tissue between the two genotypes. The volume density of intercellular spaces was significantly greater ($P = 0.001$) in Cap-9004 (.032) as compared with Cajun 1-9027 (.008).

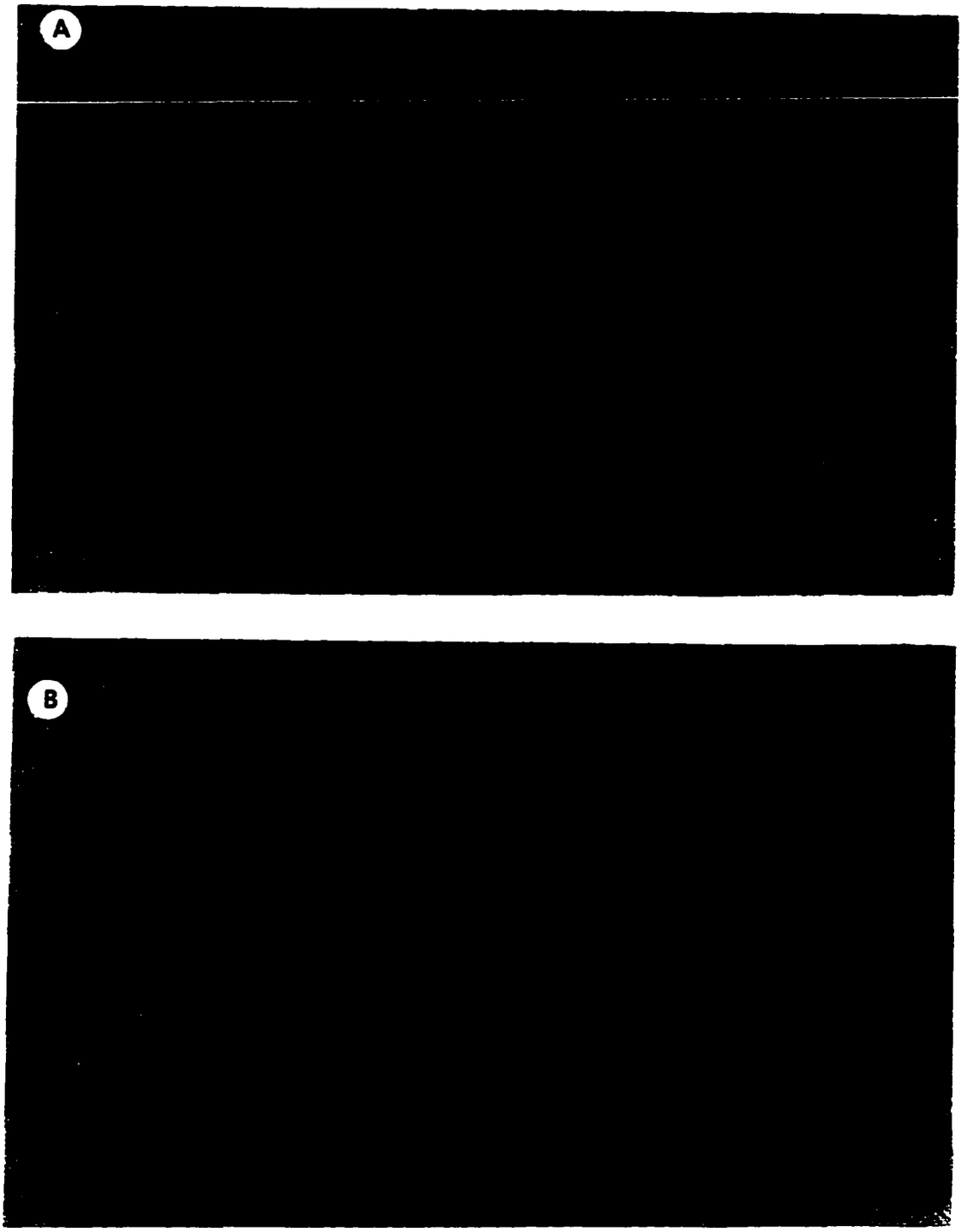

Fig. 5. Photomicrographs of longitudinal sections of cayenne pepper stained with 1.0% safranin counterstained with 1.0% alcian blue. (A) Cajun 1-9027, (B) Cap-9004. Large arrows indicate sclereids in pith. Small arrows indicate sclereids in cortex. Magnification x15.

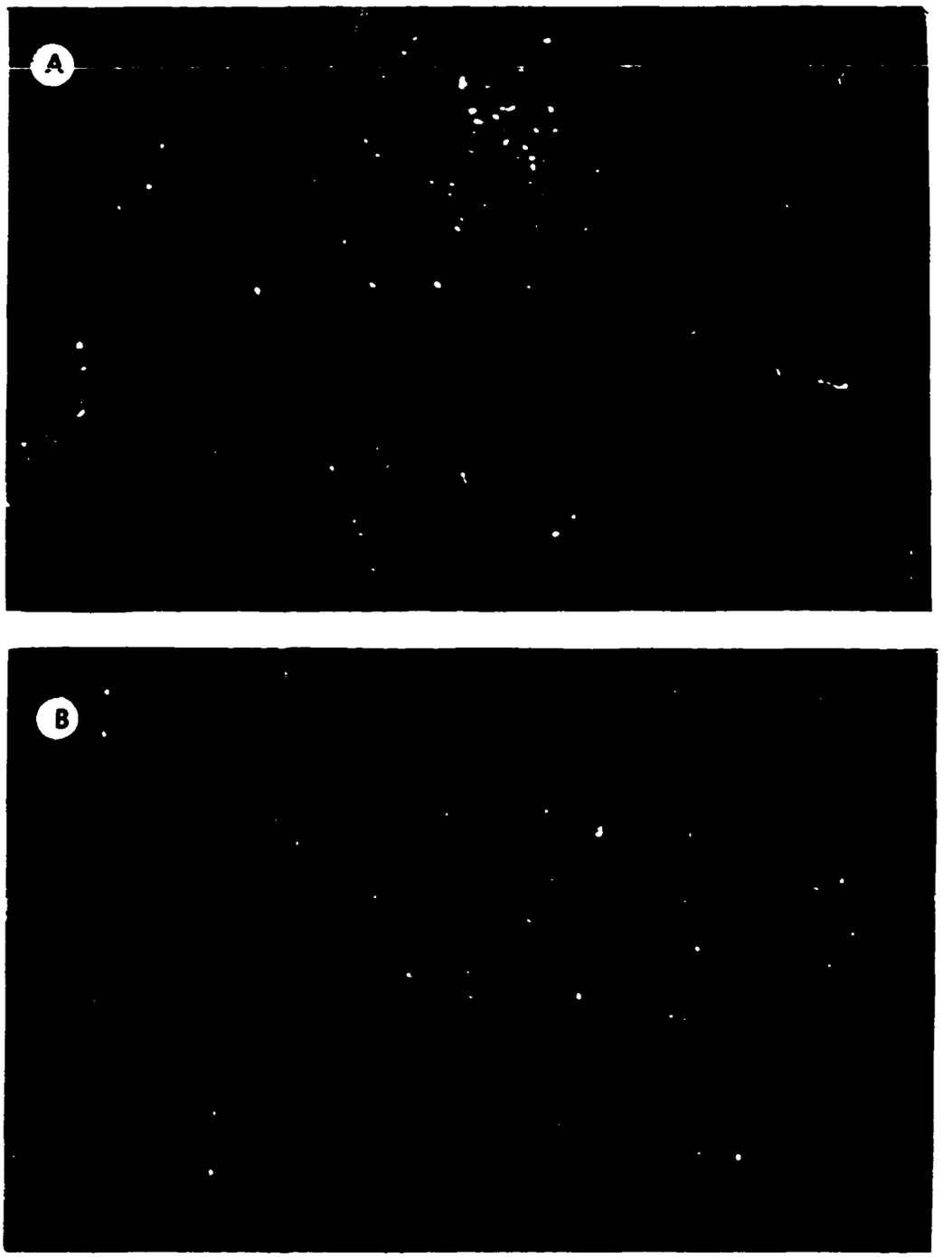

Fig. 6. Photomicrographs of longitudinal sections of cayenne pepper with polarized light (illustration of lignification). (A) Cajun 1-9027, (B) Cap-9004. Magnification x15.

Table 2. Volume density (V_v) of cell structures in the fruit-receptacle detachment area of Cajun 1-9027 and Cap-9004 (mean ± SE)

Structure	Genotype		
	Cap-9004		Cajun 1-9027
Parenchyma	.581 ± .008	***	.484 ± .008
Sclerenchyma	.294 ± .007	***	.409 ± .008
Vascular tissue	.093 ± .004	NS	.099 ± .005
Intercellular spaces	.032 ± .003	***	.008 ± .001

NS,***Nonsignificant or significant at P = 0.001, respectively.

Discussion

Differences were found in FDF for the genotypes examined in both greenhouse and field experiments. Similar results for both greenhouse and field experiments are consistent with other studies indicating genetic control (Setiamihardja and Knavel,1990; Smith,1951; Spasojevec and Webb,1971; Werner and Honma,1980). Seasonal influence on FDF for some genotypes may indicate increased physiological or developmental sensitivity to environmental factors. Wien et al. (1989) reported that the susceptibility of stress-induced abscission decreased with the age of the tissue and indicated mature fruit were most resistant. Screening methods have been developed to identify genotypes resistant to stress-induced abscission (Wien et al., 1989).

In the genotypes examined, correlations between FDF and phenotypic characters of fruit length and diameter, pedicel length, calyx diameter, length,

or scar were not consistent. This inconsistency suggests that using fruit characters would not be useful for breeding low FDF genotypes.

The location of clean detachment in Cajun-9004 was at the fruit-receptacle junction. Although only 25% of the fruit separated in this manner, it was the only consistent break. The detachment area was not composed of a distinct layer of cells, but was delineated by the juncture of the fruit and receptacle tissue just distal to the fruit-receptacle indentation. Both genotypes had this indentation which served as a useful marker. Cells in the cortex were procgressively smaller from both the receptacle and fruit toward the fruit-receptacle junction similar to the lower abscission zone in sweet cherry (Wittenbach and Bukovac, 1972). Another similarity of cayenne pepper genotypes to sweet cherry was the branching of the vascular cylinder in the detachment area. Although Wittenbach and Bukovac (1972) found no lignification of cells on either side of the abscission layer, both cayenne pepper genotypes had sclereids in the fruit-receptacle junction region. However, quantitative examination of the detachment area suggests possible genotypic differences. The volume of sclereids in Cap-9004 was less than that in Cajun 1-9027. According to Wittenbach and Bukovac (1972), this would probably result in a weakening of the detachment area and an increase in ease of detachment. Characteristically, lignin is absent, or present in only very limited amounts, in abscission zone cells and it is unlikely that the cells of the cortex of the abscission zone would undergo lignification (Baird et al., 1979).

Greater lignification throughout the detachment area in Cajun 1-9027, compared with that of Cap-9004, could contribute to the greater detachment force needed for fruit removal.

Wittenbach and Bukovac (1972) suggest the most significant component of fruit abscission may be the ripening of the fruit. In our study, fruit maturity was sequenced and collections were made at the same stage of ripeness to minimize this as a factor in differences between the two genotypes. Although firmness was not tested in our study, there was no evidence of soft flesh (Smith,1951) in either genotype at the time of sampling. Fruit from both genotypes were firm when red.

Baird et al. (1979) suggest mechanical resistances influence the ultimate degree of separation in many fruits. This could explain the difference in fruit detachment in the two genotypes studied. The increased volume of sclereids and decreased volume of intercellular space in Cajun 1-9027, as well as the arrangement and size of the cells, may be responsible for the greater force required to detach fruit compared with Cap-9004. Cajun 1-9027 may have been structurally stronger with a continuous disk of small thick walled cells through the fruit-receptacle junction region. Cap-9004 had small thick walled cells mainly in the cortex with larger thin walled cells in the pith and greater intercellular space possibly resulting in a structurally weaker detachment area in this genotype.

Future research investigating the effect of different stages of maturity and the application of abscission enhancing chemicals, such as ethephon, will help to determine the physiological changes that take place in the fruit-receptacle detachment area.

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CHAPTER 4

ETHEPHON EFFECTS ON CAYENNE PEPPER FRUIT-RECEPTACLE DETACHMENT FORCE

Introduction

Mechanical harvesting of cayenne pepper for processing could expand production areas. Past efforts to mechanically harvest red fruit have demonstrated the need to increase fruit ripening uniformity and identify cultivars that detach easily in the fruit-receptacle detachment area (Motsenbocker et al., 1992).

Ethephon (2-chloroethylphosphonic acid), an ethylene releasing compound, has been reported to effectively promote fruit ripening in various crops including pepper (Knavel and Kemp,1973; Love et al.,1971). Research on ethylene-induced color enhancement and fruit ripening has also indicated varying degrees of fruit abscission and defoliation (Sims et al.,1974; Knavel and Kemp, 1973; Cantliffe and Goodwin, 1975; Locascio and Smith, 1977; Batal and Granberry, 1982). Batal and Granberry(1982) reported that ethephon hastened and improved ripening of pimento and paprika peppers and induced defoliation and fruit abscission, especially at later stages of development. The abscission zone in most of the cultivated varieties of *Capsicum annuum*, including cayenne, occurs at the pedicel-stem junction (nondeciduous) (Cochran, 1936; Smith, 1951). In contrast, wild type peppers the separation zone occurs at the fruit-receptacle junction (deciduous) (Smith, 1951). Fruit detachment force for the fruit-receptacle junction varies when deciduous and nondeciduous genotypes of *C. annuum* are crossed (Smith, 1951; Spasojevec and Webb, 1971). Some cayenne genotypes

detach easier in the fruit-receptacle junction than others (Gersch et al., 1994; Werner and Honma, 1980). The effect of ethephon on the fruit-receptacle detachment area has not been evaluated.

Previously, two genotypes of cayenne pepper, *Capsicum annuum* L., Cajun 1-9027, which did not separate, and Cap-9004, a more readily detachable genotype, were identified that differ significantly in ease of fruit detachment at the fruit-receptacle junction (Gersch et al.,1994). This research will examine the effect of ethephon applications on the ease of fruit detachment at the receptacle and fruit junction. Concurrently, changes in the anatomical structure of the detachment area will be characterized following treatment.

Materials and Methods

Greenhouse studies. Plant Material. On 5 April 1995, seeds of two cayenne pepper genotypes (Cap-9004 and Cajun 1-9027) were sown in 72 cell trays filled with commercial media (Metro Mix 200; Grace Sierra, Milpitas, Calif.). Seedlings were transplanted on 23 May 1995, into 11.3 liter pots filled with a modified commercial media (Metro Mix 700; Marysville, Ohio) supplemented with micronutrients (Micromax; Grace Sierra, Milpitas, Calif.) 780 mg/liter, dolomitic limestone (Easy Lime; Sylacauga, Ala.) 6.26 g/liter, and slow release fertilizer (Osmocote 14N-6.2P-11.6K) 4.7 g/liter. The plants were placed on ground cover in an open greenhouse and grown under natural daylight using timed irrigation and standard cultural practices for pest control (Boudreaux et al.,1992).

Ethephon treatment. Fruit color at time of treatment was determined by using the Munsell Standard Color System (1977). Peppers were selected for treatment at mature green (5GY, 4/6) with 5-10% breaker color visible and at least 15 cm in fruit length. In cayenne pepper fruit, breaker color varies depending on the breakdown of chlorophyll. In general, fruit do not have uniform color during this transition period. Therefore, for our purposes the breaker stage was fruit that had matured past the mature green stage and did not meet the standards set for red mature fruit. Visible red pigment was at least = 5R, 4/10 for both genotypes for red fruit. Treatments were made by injection with a 1000 μl syringe (U-80; Becton-Dickinson, N.J.). Solutions tested were 500 and 750 μl liter^{-1} ethephon (Ethrel; Rhone-Poulenc, N.C.) in double distilled deionized water. In preliminary experiments, where fruit explants were placed in vials, 1000 μl liter^{-1} resulted in necrosis of the pedicel on Cajun 1-9027. Preliminary experiments indicated there were no differences in 100, 200, 300, 400 or 500 μl injections so 250 μl injections were used. The growth regulator injection technique was suggested by M. Sundberg (personal communication). Injections were made on 11 August, 1995. Ethylene solution pH was unadjusted and less than three. There were four plants per cultivar per treatment, with six fruit injected per plant. Twelve fruit per treatment were used for analyses. Previous research indicated wounding increased ethylene production in bell pepper (Saltveit, 1977). In this study two controls were used, water (double distilled deionized, unadjusted pH of

5.7) injected fruit (water control) and fruit not injected (untreated). The water injected fruit were observed to detect any evidence of wound ethylene response due to injection or pressure increase. Maximum and minimum temperature averaged 32°C ± 3° and 21°C ± 3° respectively, during the one week period from treatment to harvest. After seven days fruit were harvested and fruit detachment force was measured using a force gauge graduated in 25 g increments up to 2.5 kg (Chatillon and Sons, Kew Gardens, N. Y.). The force gauge was modified according to Werner and Honma (1980) and attached to a stand. Force was recorded in kilograms and converted to Newtons. FDF was measured on three randomly selected fruit per plant. The experiment was setup as a completely random design with factorial arrangement of treatments.

Fruit color changes were used to monitor ethylene response. Due to inherent uneven coloration of cayenne pepper fruit, visual observations of each fruit in the above treatments were used to determine the percent of color present at 24 hour time intervals after treatment. Fruit pigmentation was categorized as follows: A = mature green with less than 25% breaker color; B = 25 -100 % breaker; C = less than 50% red; D = 50 - 99% red; E = 100 % red. A mean color score per plant per day was calculated. This was accomplished by assigning a number value to the above color categories as follows: A(1), B(2), C(3), D(4), and E(5). For each day the number of fruit per plant at a particular color were multiplied by that color number. The sum of

these products for each plant was divided by the total number of fruit per plant. An analysis of variance was then performed on these mean color scores with a split plot design using plants as the main plot and time as subplot. Contrasts were also performed when appropriate.

Anatomical and Histochemical studies. Plant material. Fruits of two genotypes of cayenne pepper, Cajun 1-9027 and Cap-9004, were collected from greenhouse grown plants in 1995. Fruit were selected and treated as in the experiment above. Samples were selected randomly from these treatments for anatomical and histochemical studies after seven days.

Anatomical studies. Longitudinal tissue samples, approximately 0.6 cm x 0.3 cm, were prepared to include the detachment region, located at the fruit-receptacle junction. Approximately equal portions of fruit and pedicel were included. Samples were placed immediately into 5:5:90, 37% formaldehyde: glacial acetic: 70% ethanol (FAA), by volume, and subjected to a mild vacuum for 24-48 hours.

Light microscopy (LM). FAA- fixed samples were dehydrated and embedded in paraffin using standard techniques (Jensen, 1962). Sections were cut at 5-10 μm using disposable blades on a rotary microtome. To analyze general cellular structure, sections were stained with 0.5% aqueous toluidine blue or 1.0% safranin counterstained with 1.0% aqueous alcian blue. For histochemical studies, slides were stained with ruthenium red for pectin localization, periodic acid-Schiff's (PAS) reagent for detection of insoluble

polysaccharides, iodine-potassium iodide (IKI) to observe starch distribution, and phloroglucinol and HCl for lignin detection (Jensen, 1962). Polarized light was used to confirm the last two tests (Berlyn and Miksche, 1976).

Results

Greenhouse studies. Ethephon treatments did not significantly ($P = 0.12$) affect fruit detachment force for either genotype (Table 3). There were no significant differences ($P = 0.10$) between genotypes for a particular treatment.

Table 3. Effect of ethephon on fruit detachment force (FDF) of 2 cayenne pepper genotypes at the fruit-receptacle detachment area.

		Genotypes			
		Cap-9004		Cajun 1-9027	
Treatment	Concn (mg/liter)	No. of fruit	FDF(N)[z]	No. of fruit	FDF(N)[z]
Untreated	0	12	15.29 ± 2.45	12	16.95 ± 3.72
H_2O Control	0	12	16.86 ± 2.45	12	18.13 ± 4.21
Ethephon	500	12	17.44 ± 2.55	12	18.03 ± 3.53
Ethephon	750	12	17.73 ± 2.25	12	17.25 ± 3.43

[z]Mean ± SE

The degree to which ethephon stimulated fruit color was dependent on the genotype and independent of the concentration of ethephon used. Contrasts were used to determine differences in Cajun 1-9027 due to significant interactions between treatments over time. There were significant differences in ethephon treated fruit and the untreated and water controls by the first day after treatment for genotype Cajun 1-9027 (Fig. 7). The ethephon treated fruit

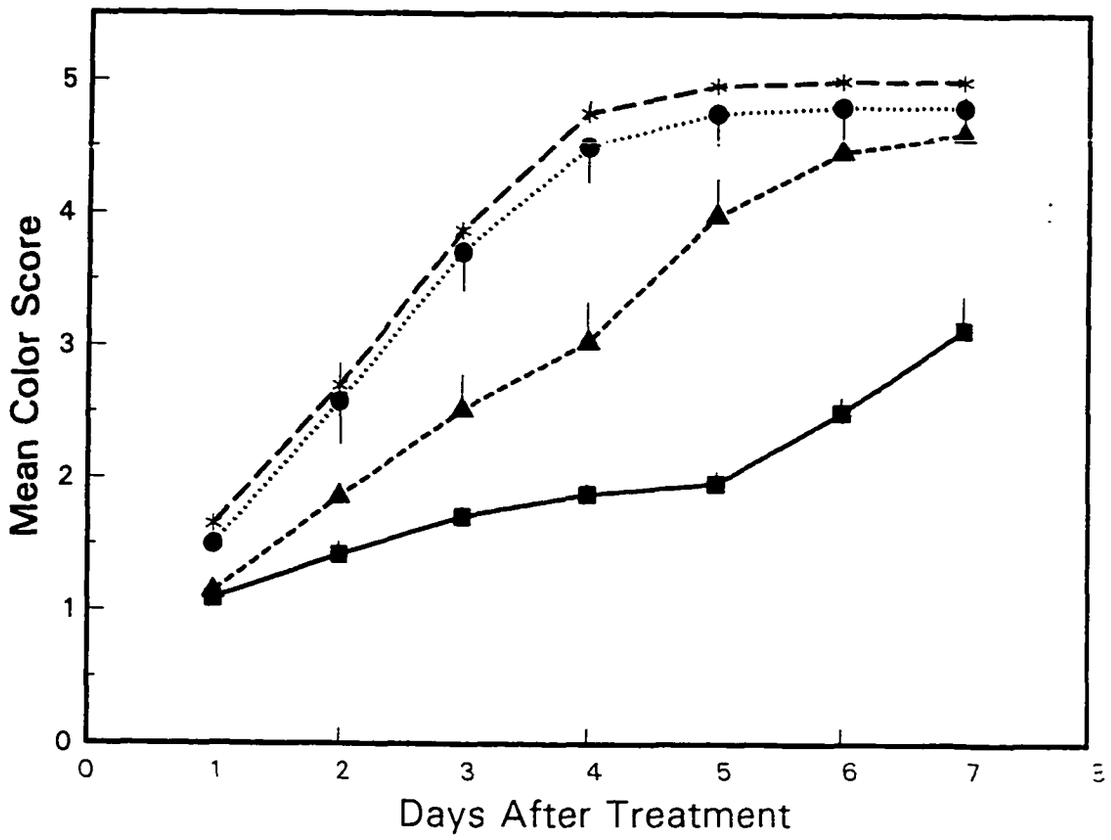

Fig. 7. The effect of ethephon on color development in Cajun 1-9027. untreated (■), water control (▲), ethephon 500 μl liter^{-1} (●), ethephon 750 μl liter^{-1} (✳). Vertical lines equal 1 SE (n = 24). Bars smaller than symbols are not shown.

and the water control had significant increases in mean color score for the first four days and this trend continued for the water treated control through day six. After four days, ethephon treated fruit had a mean color score ≥ 4.5 and did not change significantly. There were significant differences between the ethephon treated fruit and the water control by day two and these differences continued through day five. There were also significant differences between untreated fruit and the other three treatments from day three until the end of

the study. After day five the rate of color change increased significantly for the untreated control generating a mean color score near 3.1 by day seven. By day six there were no differences between the water control and the ethephon treated fruit although the untreated fruit were significantly different from the treated fruit.

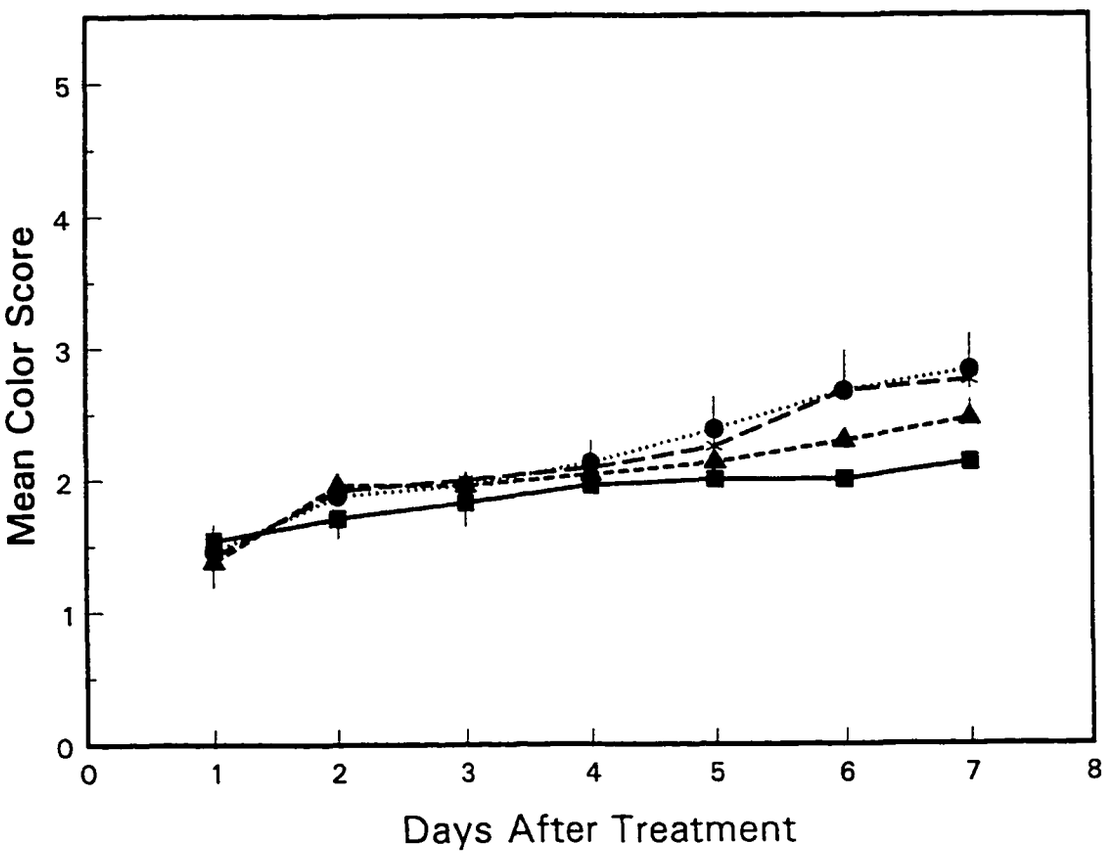

Fig. 8. The effect of ethephon on color development in Cap-9004. untreated (■), water control (▲), ethephon 500 μl liter^{-1} (●), ethephon 750 μl liter^{-1} (∗). Vertical lines equal 1 SE (n = 24). Bars smaller than symbols are not shown.

There were no significant interactions between treatments and days in mean color score for Cap-9004. Days are significant over all. Contrasts were performed to compare the means for each treatment. There was a significant difference ($P < 0.0001$) between the untreated fruit and all treatments including the water control(Fig. 8). A significant difference ($P < 0.05$) was also noted between the water control and the two ethephon treatments. The ethephon treatments did not differ significantly ($P < 0.68$). No treatments produced a mean color score higher than or equal to three during this time interval for Cap-9004.

Anatomy and histochemistry of detachment area. Localization data for insoluble polysaccharides and pectins revealed no change through the detachment area of either genotype in comparison to the adjacent fruit and receptacle tissue with ethephon treatments (data not shown). Cajun 1-9027 stained intensely in all tests indicating a dense cell structure of thick cell walls in small closely packed cells. In comparison, Cap-9004 stained less intensely suggesting a less dense cell structure (Chapter 3).

Localization of starch was not affected by ethephon treatments (data not shown). Tests indicated little or no starch in the area of detachment. Starch was present in the receptacle and the fruit tissue of both genotypes for all treatments.

Lignified cells were present throughout the fruit-receptacle junction in Cajun 1-9027 with no apparent change due to ethephon treatment (data not

shown). In all tests there was less lignification in the detachment area in Cap-9004 than in Cajun 1-9027 but no apparent change due to ethephon treatment.

Discussion

Penetration of ethylene releasing compounds into plants and their subsequent movement within plant tissues can influence their effectiveness (Kays and Beaudry, 1987). Penetration is generally less in fruit tissue than in vegetative tissue. Temperature and relative humidity can affect ethylene evolution rate as a result of ethephon decomposition and extent of tissue penetration (Beaudry and Kays, 1987; Bukovac et al., 1969; Olien and Bukovac, 1982; Warner and Leopold, 1969). Denny and Martin (1994) cited no difference in ethephon penetration as a function of solution pH. They noted tissue type was more important than solution pH for determining the extent of penetration. Research with olive indicated that duration of ethylene exposure from ethylene releasing compounds critically governs plant response (Lang and Martin, 1987). We chose injection because we were able to target the organ of choice, ensure penetration, and increase exposure time. Once inside the fruit we expected the ethephon solution pH to change, because the intercellular pH is approximately 4.5, thus releasing ethylene (Yang,1969).

Reports indicate applications of 500 μl liter^{-1} ethephon solution increase the number of abscised pepper fruit (Cantliffe and Goodwin, 1975; Batal and Granberry, 1982). Denny and Martin (1994) reported a significant response

to olive fruit removal force with 600 μl liter^{-1} ethephon solution after five days. These reports and the fact that 1000 μl liter^{-1} caused adverse effects in preliminary experiments with Cajun 1-9027 led us to use 500 and 750 μl liter^{-1}. No differences were observed in fruit detachment force for either genotype with these concentrations. Fruit physiological age influences abscission and fruit separation by ethephon (Batal and Granberry, 1982). In our study, the fruit were selected mature green with some breaker color showing (5-10%) to ensure they were at or past the mature green stage. Beaudry and Kays (1988) suggest that induction of a particular plant organ requires that the sensitivity of the target organ to the ethylene releasing compound be sufficiently high. This sensitivity for induction of abscission in the fruit-receptacle detachment area is not apparent for fruits of these pepper genotypes under these experimental conditions.

Both genotypes display evidence of color response due to ethylene concentration although there are genotypic differences. Cajun 1-9027 had an increased rate of ripening at this physiological stage of development which is illustrated by observing the control increase in mean color score from near one to greater than three after day seven (Fig. 1). Ethephon treated fruit of genotype Cap-9004 did not show this degree of change in mean color score over the seven day period. This may mean our estimate of physiological maturity for Cap-9004 was not adequate or this genotype has a slower response time. Harvested pimento peppers, beyond the breaker stage,

develop normal red color when treated with ethephon (Lockwood and Vines, 1972). However, harvested mature green pimento or bell peppers failed to develop acceptable red color when treated with ethephon or ethylene (Knavel and Kemp, 1973; Lockwood and Vines, 1972). Reports indicate pepper cultivars differ in their response to ethephon (Cantliffe and Goodwin, 1975). One cultivar, 'Shepherd', responded to concentrations as low as 10 μl liter^{-1} applied twice while 'Staddon's Select' did not respond to concentrations as high as 750 μl liter^{-1}.

Both genotypes (Cap-9004 and Cajun 1-9027) responded to water injections by a ripening response suggesting the effect of wound ethylene. Mature green bell pepper fruit (*C. annuum* L. cv. Yolo Wonder) exhibited a non-climacteric pattern of ethylene and carbon dioxide production during normal ripening or exposure to propylene (Saltveit, 1977). However, wounding excised plugs of ovary wall tissue caused an increase in carbon dioxide production in one day and an increase in ethylene production by day two. A similar pattern of events occurred in green tomato (*Lycopersicon esculentum* Mill.) (Yu and Yang, 1980), different ripening stages of tomato (Kende and Boller, 1981) and preclimacteric cantaloupe (*Cucumis melo* L.) (Hoffman and Yang, 1982). Ethylene, ACC, and ACC synthase activity all increased manyfold a few hours after wounding and even after cessation of ethylene production, ACC and ACC synthase levels remained high. The

response to water injections in Cajun 1-9027 was more evident than in Cap-9004 corresponding with a higher color response in general.

The location of clean detachment in Cajun-9004 is at the fruit-receptacle junction (Chapter 3). The detachment area is not composed of a distinct layer of cells, but delineated by the juncture of the fruit and receptacle tissue just distal to the fruit-receptacle indentation. Both genotypes have this indentation, which serves as a useful anatomical marker. The cortical cells in this region are progressively smaller cells than those more proximal in the receptacle and distal in the fruit. This is similar to the lower abscission zone in sweet cherry (Wittenbach and Bukovac, 1972). Although Wittenbach and Bukovac (1972) reported no lignification of cells on either side of the abscission layer, both genotypes of cayenne pepper have sclereids in the fruit-receptacle junction region. Examination of the detachment area, however suggests possible genotypic differences. The volume of sclereids in Cap-9004 is less than that in Cajun 1-9027 (Chapter 3). According to Wittenbach and Bukovac (1972), a decrease in sclereids would probably result in a weakening of the detachment area and an increase in ease of detachment. Characteristically, lignin is absent, or present in only very limited amounts, in abscission zone cells and it is unlikely that the cells of the cortex of the abscission zone would undergo lignification (Baird and Webster, 1979). Greater lignification throughout the detachment area in Cajun 1-9027, compared with that of Cap-9004, could contribute to the greater detachment

force needed for fruit removal. Lignification was not noticeably different due to any ethephon treatment for either genotype.

Wittenbach and Bukovac (1972) suggested the most significant component of fruit abscission may be fruit ripening. In our study, the index of fruit maturity was based on fruit color to ensure the same stage of ripeness at injection. The assumption was that fruit color coincided with physiological maturity. The color response indicates that at the time of harvest, three treatments of Cajun 1-9027 were red and none of the treatments of Cap-9004 were fully red. Since the untreated control in both genotypes had the lowest FDF the modifications that brought about these color changes did not appear to enhance ease of fruit detachment.

In conclusion, our data suggests that cells in the detachment area are not sensitive to ethylene induced detachment since the ethylene releasing compound initiates a color response with no differences in FDF between treated and untreated fruit for either genotype. In addition, the histochemical study indicated no evidence of an AZ with or without ethephon treatment. Since, no signs of an ethylene induced AZ have been indicated, it appears, that fruit separation is due to mechanical forces.

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CHAPTER 5

A QUANTITATIVE STUDY OF SCLEREID DIFFERENTIATION IN THE FRUIT-RECEPTACLE JUNCTION OF CAYENNE PEPPER DURING FRUIT DEVELOPMENT

Introduction

The fruit abscission zone in most of the large cultivated varieties of *Capsicum annuum*, including cayenne, occurs at the pedicel-stem junction (nondeciduous) (Cochran, 1936; Smith, 1951). In small wild-type fruit the separation occurs at the fruit-receptacle junction (deciduous) (Smith, 1951). Fruit detachment force at the fruit-receptacle junction differs in the progeny of crosses of deciduous and nondeciduous genotypes of *Capsicum annuum* (Smith, 1951; Spasojevec and Webb, 1971). Cross pollination in peppers produces heterogeneity in fruit characteristics in general (Odland and Porter, 1941). The force required to detach fruit in the fruit-receptacle junction differs due to genotype for cayenne peppers (Gersch et al., 1994; Werner and Honma, 1980).

Previous research indicated that two cayenne genotypes differed significantly in the volume of sclereids and the ease of fruit detachment for mature red fruit (Gersch et al., 1994). Both cayenne genotypes have sclereids in the fruit-receptacle junction extending into the fruit from just beneath the outer epidermis throughout the pericarp. The presence of sclereids in the fruit-receptacle detachment area of cayenne pepper is believed to increase the mechanical force needed to separate the fruit from the calyx (Chapter 3). Ease of fruit detachment is an important characteristic for cayenne as removal of the green calyx and woody stem is essential for processed sauce to retain good color and quality.

Sclerenchyma and collenchyma are supporting tissues consisting of thick walled cells. Based on previous work (Munting, 1974; Chapter 3) small and large fruited varieties of *Capsicum annuum* appear to differ in types of supportive tissue. Two small fruited varieties of *Capsicum annuum* contain four layers of collenchyma under the fruit outer epidermis (Munting, 1974). Interior to the collenchyma were 16 layers of parenchyma cells with sclerenchyma found only in the inner epidermal layer. Collenchyma cells have relatively soft, pliable, nonlignified primary walls, whereas sclerenchyma cells have hard, more rigid, secondary walls, which are commonly lignified (Esau, 1977).

There are two basic types of sclerenchyma, sclereids and fibers. Sclereids may differentiate in many areas and the developmental signal for differentiation is not known. They differentiate from primary meristems, various types of parenchyma cells in the leaves, cortex or pith, and from secondary meristems (Esau, 1977). Sclerenchyma cells are often scattered among cells of other types, individually or in small groups.

Like sclereids, the water conducting cells of xylem, and associated parenchyma cells, are lignified. Ye and Varner (1995) predicted that in the future, alteration of lignin content and composition of fibers and sclereids without perturbing the water-conducting xylem cells may be possible. A better understanding of the differentiation of sclereids is needed before selective lignification will be possible.

The purpose of this study was twofold: 1) to quantitatively describe the differences in sclereid volume in the fruit-receptacle region between two genotypes of cayenne pepper during development and 2) to determine at which developmental stage such differences first could be observed.

Materials and Methods

Macroscopic morphology studies. Two cayenne pepper genotypes, Cap-9004 and Cajun 1-9027, were used. On 5 November 1995, all fruit were removed from greenhouse grown plants to encourage flowering. Beginning 12 November, 1995, flowers at anthesis were marked twice per week and for the next thirteen weeks. On 11 February all marked fruit were harvested and length and width were measured. Fruit length was measured with a metric ruler as the distance from pedicel attachment at the calyx to its apex. Maximum fruit width was measured with vernier calipers.

Microscopic morphology studies. Sample preparation. Five fruit per genotype from each of the above dates were sampled. Longitudinal tissue samples, approximately 0.6 cm x 0.3 cm, were prepared to include the fruit-receptacle junction. Approximately equal portions of fruit and pedicel were included. Samples were placed immediately into 5:5:90, 37% formaldehyde: glacial acetic: 70% ethanol (FAA), by volume, and subjected to a mild vacuum for 24-48 hours.

Samples were dehydrated and embedded in paraffin using standard techniques (Jensen, 1962). Sections were cut at 7 μm using disposable

blades on a rotary microtome. To analyze general cellular structure, sections were stained with 1.0% safranin counterstained with 1.0% alcian blue. Polarized light was used to confirm lignin detection (Berlyn and Miksche, 1976).

Quantitative analysis. For each sampling date, one block of each genotype was randomly chosen for quantitative analysis. The median longitudinal section from each of these was analyzed. A micrometer was used to define a rectangular sampling area on each slide and random sampling was performed using the x and y scales on the microscope stage. The width of the rectangle was determined by the fruit-receptacle indentation on each fruit. To ensure the same representative area on each fruit was used in data collection, the length of the rectangle was calculated to maintain a length to width ratio based on mature fruit. The area on mature fruit was equivalent to a 2 mm length on either side of the fruit-receptacle indentation. For each area, four or five random samples were scored using an ocular grid reticle at a magnification of 400x. Point counts (64 point grid) were recorded for cells and intercellular spaces in each sample (Toth, 1982).

Stereological calculations for volume density (or fraction) of sclereids (V_v(scl)) is defined by the ratio of the volume of sclereids (V(scl)) (mm^3) to the fruit-receptacle volume (V(f-r)) (mm^3):

$$V_v(scl) = V(scl)/V(f\text{-}r)$$

Volume density was estimated by point-counting (Weibel, 1979).

The experiment was arranged in a split-plot design with genotypes as the main plot and harvest dates as subplot treatments. Measurement data were subjected to analysis of variance and contrasts were performed.

Results

Macroscopic morphology. Fruit of both genotypes reach maturity at about 13 WAA. Fruit length is not significantly different for the two genotypes until the last two weeks of development (Fig. 9).

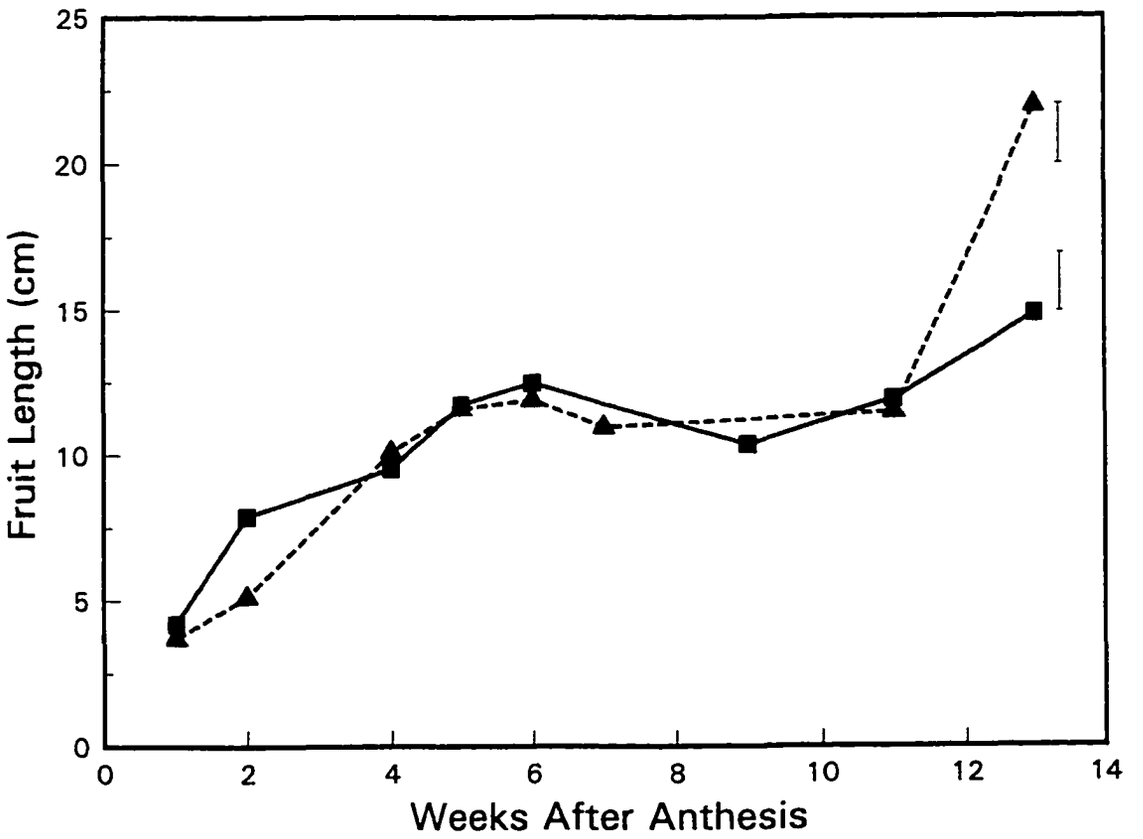


Fig. 9. Relative fruit length. (■) Cap-9004 (▲) Cajun 1-9027. Vertical bars represent maximum SE (n = 5).

Cajun 1-9027 mean fruit length increases significantly from less than 12 cm to greater than 21 cm between week 11 and 13. Fruit length was only significantly different between the two genotypes at 13 WAA.

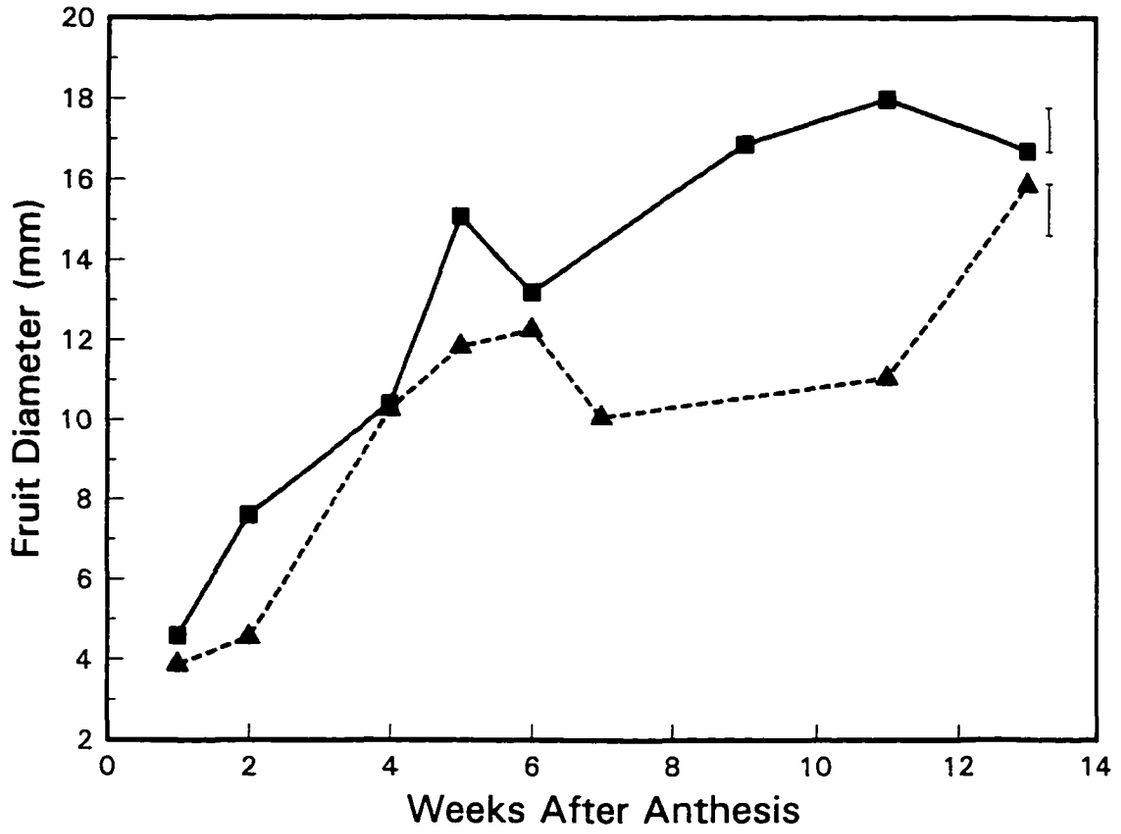


Fig. 10. Relative fruit diameter. (■) Cap-9004 (▲) Cajun 1-9027. Vertical bars represent maximum SE (n = 5).

Of the thirteen weeks sampled, weeks 1,2,4,5,6,11, and 13 had enough (four or five) fruit of both genotypes for analysis. Cajun 1-9027 also had enough fruit during week 7 and Cap-9004 had enough fruit during week 9. The lower number of fruit in the middle of the experiment was probably due to low light intensity in mid-winter. The dropping of buds, blossoms, and partially mature

fruit in pepper is a general phenomenon due to numerous factors (Cochran, 1936).

There were significant interactions between varieties and harvest for fruit diameter so contrasts were used to determine differences. Fruit diameter increased significantly for both genotypes in the first five weeks of growth (Fig. 10). There was no significant increase in fruit diameter between week six and week 13 for Cap-9004. Cajun 1-9027 fruit diameter did not change significantly between week five and week 11 but increased significantly between week 11 and week 13. Although fruit diameter was significantly different at 11 WAA between the two genotypes, it was approximately 15 mm at 13 weeks and not significantly different.

Contrasts were used to determine the following differences because there were significant interactions between varieties and harvests. The fruit length/diameter was not significantly different for Cajun 1-9027 from one WAA to 11 WAA, but increased significantly between week 11 and week 13 (Fig. 11). In Cap-9004 the fruit length/diameter was not significantly different between week one and week six although it decreased significantly between week six and week nine. After week nine there was no significant change in the length/diameter for Cap-9004. The length/diameter between the two genotypes was only significantly different at 11 WAA and 13 WAA.

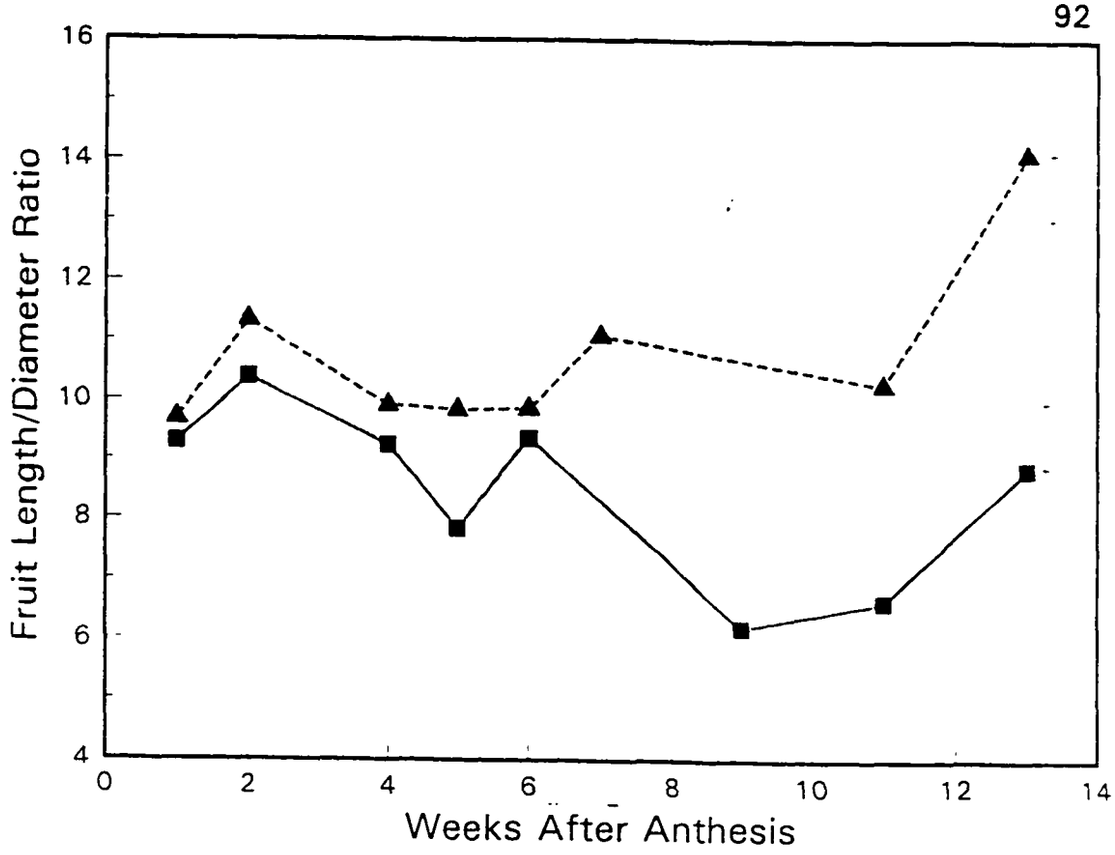


Fig. 11. Relative fruit length/diameter. (■) Cap-9004 (▲) Cajun 1-9027 Vertical bars represent maximum SE (n = 5).

Microscopic morphology studies. Anatomy and histochemistry. At one WAA no sclereids were evident in the fruit-receptacle indentation area in either genotype (Fig 12). Birefringent areas are restricted to xylem tissue and scattered crystals. At two WAA sclereid development was initiated in the peripheral parts of the hypanthium in both genotypes (Fig 13). By four WAA patches of sclereids were recognizable in the pericarp/cortex region in both genotypes (Fig. 14a and b). By week 13 extensive regions of sclerenchyma extended into the calyx and fruit of both genotypes (Fig 14c and d). Genotypic differences in sclereid differentiation were observed in the fruit-receptacle indentation region. In Cajun 1-9027 sclereids typically

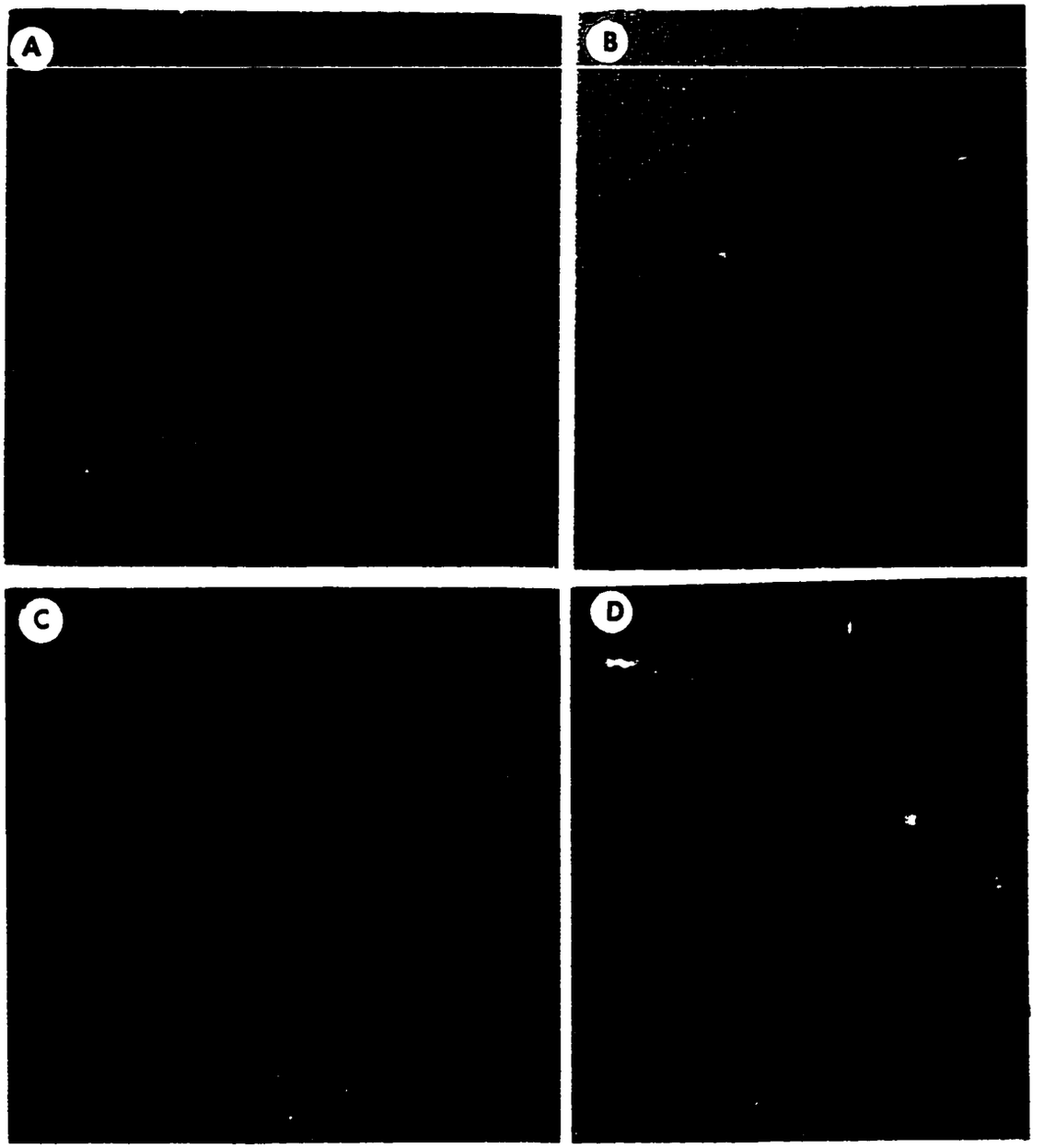


Fig. 12. Photomicrographs of longitudinal sections with polarized light of cayenne pepper fruit one WAA with no sclereids evident. Cajun 1-9027 (A) Magnification = x7.5. (B) Higher magnification view of boxed area Magnification = x40. Cap-9004 (C) Magnification = x7.5. (D) Higher magnification view of boxed area Magnification = x40. Abbreviations: calyx (C), fruit (F), and indentation (I).

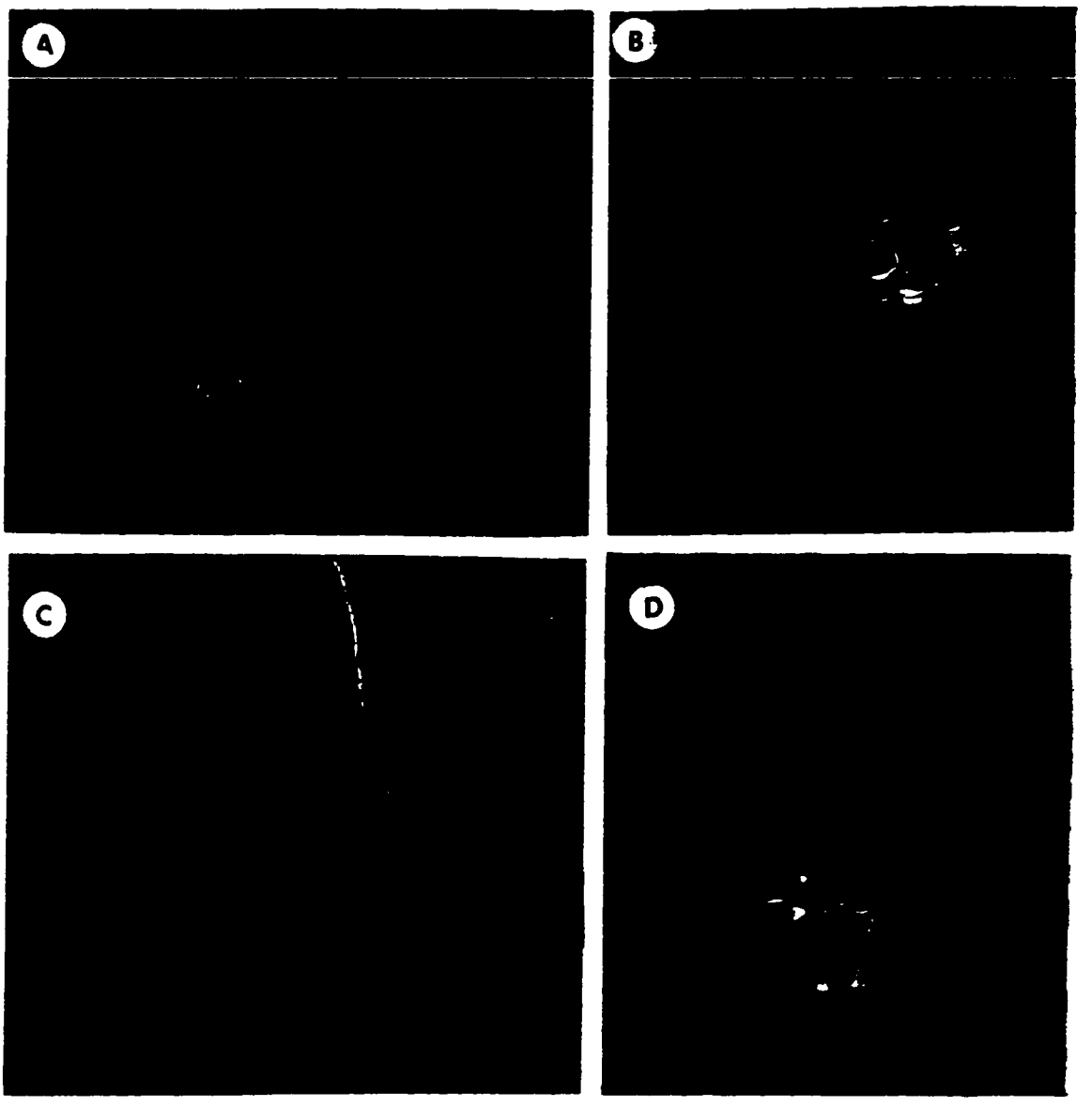
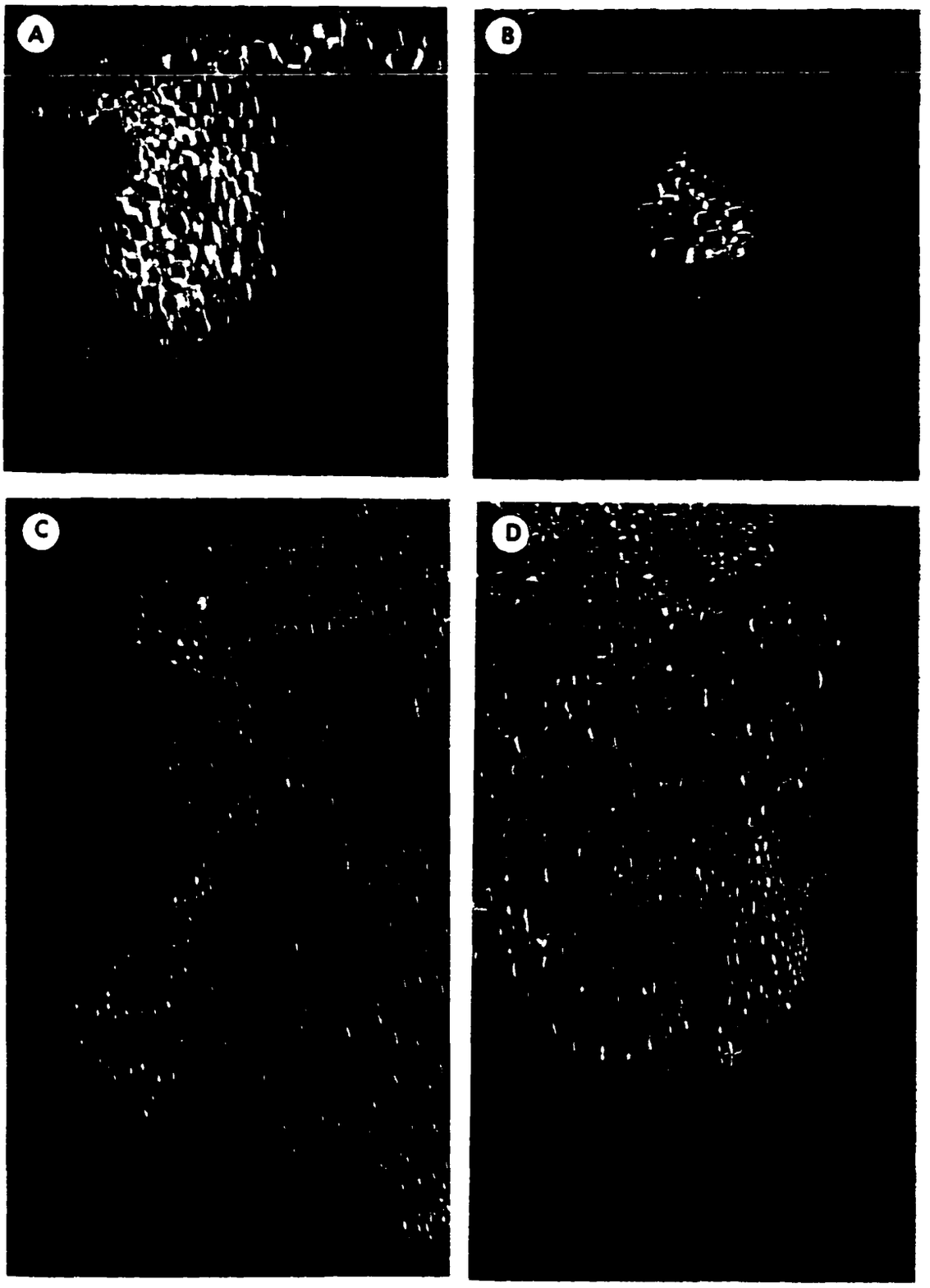


Fig. 13. Photomicrographs of longitudinal sections with polarized light of cayenne pepper fruit two WAA with sclereids visible. Cajun 1-9027 (A) Magnification = x7.5. (B) Higher magnification view of boxed area Magnification = x200. Cap-9004 (C) Magnification = x7.5. (D) Higher magnification view of boxed area Magnification = x200. Abbreviations: sclereids (S), parenchyma (P), and indentation (I).

extended into the fruit 25-30 rows of cells past the indentation (Fig 14c). Cell

to cell contact throughout the fruit-receptacle junction was sclereid to sclereid.

Fig. 14. Photomicrographs of longitudinal sections with polarized light of cayenne pepper fruit four (A and B) and 13 (C and D) WAA containing the fruit-receptacle indentation region. Magnification = x100. (A) Cajun 1-9027, (B) Cap-9004, (C) Cajun 1-9027, (D) Cap-9004 Abbreviations: sclereids (S), parenchyma (P), and indentation (I).



Sclereids in Cap-9004 generally only extended past the indentation 10 - 15 rows of cells (Fig 14d). The transition from sclerenchyma to parenchyma was near or just below the fruit receptacle indentation.

Quantitative analysis. Interactions between variety and harvest were significant for sclereid volume density so contrasts were performed to detect differences. Sclereid volume density increased significantly between week six and week nine for Cap-9004. However, differences were not significant before week six or after week nine between consecutive harvests (Table 4).

Table 4. Sclereid volume density (V_v) in the fruit-receptacle detachment area of Cajun 1-9027 and Cap-9004 (mean ± SE).

Weeks after anthesis	Genotype		
	Cap-9004		Cajun 1-9027
1	0	NS	0
2	0	NS	0
4	.073 ± .054	NS	.037 ± .020
5	.196 ± .046	NS	.142 ± .041
6	.082 ± .023	NS	.231 ± .059
7	----------	----	.218 ± .039
9	.334 ± .066	----	----------
11	.267 ± .095	NS	.401 ± .076
13	.348 ± .029	**	.583 ± .034

NS,**Nonsignificant or significant at P = 0.01, respectively.

Sclereid volume density increased after week four for Cajun 1-9027 through week 13 although differences were only significant between week four and 11 and week four and 13. Sclereid volume density was significantly different (P = 0.005) between the two genotypes only at 13 weeks.

Discussion

Increase in fruit length, for both genotypes, appears to be a typical sigmoid curve (Munting, 1974; Coome, 1976). Both genotypes developed to a red mature stage at approximately 13 WAA. In Cajun 1-9027 the fruit length increased more than the diameter such that the length/diameter is 10.5 for fruit at one WAA but 13.8 for mature fruit at 13 WAA (Fig. 11). Cap-9004 fruit length increased proportional to the width during development. Fruit length/diameter was 9.1 for one WAA fruit and 8.9 for mature fruit.

In both genotypes there was a zone of very small cells near the base of the ovary (Fig. 12) which was an active site for early sclereid development (Fig. 13). A trend of increasing sclereid volume density in Cajun 1-9027 throughout maturity was evident (Table 4). However, in Cap-9004 sclereid volume density increased to week nine then leveled off. There were no significant differences in sclereid volume density between the genotypes throughout much of fruit development.

Sclereids began to develop in fruit at two WAA and were clearly evident by week four in both genotypes (Fig. 13 and 14, Table 4). In pear, differentiation of sclereids starts about two weeks after anthesis and spreads

centrifugally through developing fruit tissue (Sterling, 1954). Larger isolated sclereids form first, but subsequently sclereid formation appears so that adjoining cells are stimulated to become sclerefied. This may also be the case with *Capsicum* since soon after differentiation large sclerefied areas form (Fig. 13 and 14).

In summary, this study presents evidence of sclereid differentiation and development in *Capsicum annuum* fruit. Sclereid differentiation occurred in the fruit-receptacle junction in the pericarp/cortex region around two WAA for both genotypes. Differences were highly significant by week 13 ($P = 0.005$). Saltveit (1977) proposed that domestication of some *Capsicum* species led to the selection of pendulant, nonabscising fruit, while wild pepper and other domestic cultivars have erect, deciduous fruit. As this selection took place, the change from small erect to larger pendulant fruit may have resulted in more structural tissue. Development of more structure could have led to sclereid differentiation and inadvertently sclereid selection. Sclereid induction has been associated with wounding (Dircks and Grange, 1983; Lev-Yadun, 1994). Higher stress in this fruit-receptacle region as fruit size increased with development may have also been a stimulus for sclereid development. This study did not answer what triggers sclereid differentiation but sclereid development over time has been identified. This knowledge may be useful in future research to selectively control lignification.

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CHAPTER 6

SUMMARY AND PROSPECTUS

Fruit detachment at the fruit-receptacle junction was investigated in two genotypes of cayenne pepper (*Capsicum annuum* L.). The usefulness of ethephon application as a potential tool in ease of detachment between the receptacle and the fruit was also investigated. In addition, anatomical and histochemical studies were examined to further elucidate the fruit-receptacle detachment area in each genotype.

Differences in FDF were found for the genotypes examined in both greenhouse and field experiments (Chapter 3). Similar results for both greenhouse and field experiments are consistent with other studies indicating genetic control of fruit detachment in pepper (Setiamihardja and Knavel, 1990; Smith, 1951; Spasojevec and Webb, 1971; Werner and Honma, 1980).

In the genotypes examined, correlations between FDF and phenotypic characters of fruit length and diameter, pedicel length, calyx diameter, length, or scar were not consistent. This inconsistency suggests that using fruit characters would not be useful for breeding low FDF pepper genotypes.

The location of clean detachment in Cajun-9004 was at the fruit-receptacle junction (Chapter 3). Although only 25% of the fruit separated in this manner, it was the only consistent break. The detachment area was not composed of a distinct layer of cells, but was delineated by the juncture of the fruit and receptacle tissue just distal to the fruit-receptacle indentation. Both genotypes had this indentation and progressively smaller cells in the cortex from the receptacle and fruit toward the fruit-receptacle junction. This anatomical

feature in pepper is similar to the anatomical description of the lower abscission zone in sweet cherry (Wittenbach and Bukovac, 1972).

Both genotypes of cayenne pepper had sclereids in the fruit-receptacle junction region (Chapter 3 and 5). However, examination of the detachment area indicates genotypic differences. The volume of sclereids in Cap-9004 was less than that in Cajun 1-9027. According to Wittenbach and Bukovac (1972), this would probably result in a weakening of the detachment area and an increase in ease of detachment. Characteristically, lignin is absent, or present in only very limited amounts, in abscission zone cells and it is unlikely that the cells of the cortex of the abscission zone would undergo lignification (Baird et al., 1979). Greater lignification throughout the detachment area in Cajun 1-9027, compared with that of Cap-9004, could contribute to the greater detachment force needed for fruit removal.

Baird et al. (1979) suggests mechanical resistances influence the ultimate degree of separation in many fruits. This could explain the difference in fruit detachment in the two genotypes studied. The increased volume of sclereids (Chapter 3 and 5) and decreased volume of intercellular space (Chapter 3) in Cajun 1-9027, as well as the arrangement and size of the cells, may be responsible for the greater force required to detach fruit of this genotype compared with Cap-9004. Cajun 1-9027 may be structurally stronger with a continuous disk of small thick walled cells through the fruit-receptacle junction region. Cap-9004 had small thick walled cells mainly in the cortex with larger

thin walled cells in the pith and greater intercellular space possibly resulted in a structurally weak fruit-receptacle detachment area in this genotype.

Our data suggests that cells in the fruit-receptacle detachment area are not sensitive to ethylene induced detachment since the ethylene releasing compound, ethephon, resulted in no differences in FDF between treated and untreated fruit for either genotype (Chapter 4). No differences were observed in fruit detachment force for either genotype when treated with 500 and 750 μl liter^{-1} ethephon compared to untreated fruit. In contrast, ethephon enhanced fruit coloration in both genotypes although there were differences due to genotype. Cajun 1-9027 had an increased rate of ripening at the mature fruit stage used in this study. Both genotypes (Cap-9004 and Cajun 1-9027) responded to water injections (water control treatment) by a ripening response suggesting the effect of wound ethylene. The response to water injections in Cajun 1-9027, however, was more pronounced than in Cap-9004 corresponding with an enhanced color response in general. Lignification in the fruit-receptacle detachment area was not noticeably changed with any ethephon treatment for either genotype. There was no evidence in the histochemical studies of any structural changes as a result of the ethylene treatments in either genotype. In addition, the histochemical study indicated no evidence of an AZ with or without ethephon treatment. Since, there was no evidence of an ethylene induced AZ in our studies, it appears, that

differences in cayenne pepper fruit detachment are due to structural differences and fruit separation is due to mechanical forces.

The present experiments lay a foundation for future work which may develop methods of easier hand or mechanical fruit removal. This study presents the first direct evidence of sclereid differentiation in *Capsicum annuum* fruit. Sclereid differentiation occurred around week two for both genotypes in the fruit-receptacle junction in the pericarp/cortex region (Chapter 5). Differences were significant by week 13 ($P = 0.005$). Saltveit (1977) proposed that domestication of some *Capsicum* species led to the selection of pendulant, nonabscising fruit, while wild pepper and other domestic cultivars have erect, deciduous fruit. As this selection took place, the change from small erect to larger pendulant fruit may have resulted in more structural tissue. This increase in structure could have led to sclereid development and inadvertently sclereid selection. Sclereid induction has also been associated with wounding (Dircks and Grange, 1983; Lev-Yadun, 1994). Stress in this fruit-receptacle region as fruit size increased may have also been a stimulus for sclereid differentiation. This study did not answer what triggers sclereid differentiation, but development over time has been identified. The results of this work and research in the future on lignification may lead to successful control. Like sclerenchyma, water conducting cells of the xylem and associated parenchyma cells have secondary walls. Ye and Varner (1995)

predict that in the future, alteration of lignin content and composition of fibers and sclereids without perturbing the water-conducting cells may be possible.

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VITA

Kay P. Gersch was born Kay Delight Purvis on 17 January, 1950, in Independence, Louisiana. She attended elementary and secondary school at Fourth Ward and Albany, Louisiana. She completed her Bachelor of Science degree in chemistry education at Southeastern Louisiana University in Hammond (1977), and her Master of Science in biology education at University of Southern Mississippi in Hattiesburg (1991). In August 1992, she entered the graduate schoool in the Department of Horticulture, Louisiana State University for a doctoral program. In August 1996, the Doctor of Philosophy Degree will be conferred.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Kay P. Gersch

Major Field: Horticulture

Title of Dissertation: Analysis of the Influence of Genotype on Cayenne Pepper Fruit-Receptacle Detachment

Approved:

Carl E Motsenbocker
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

May 3, 1996